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VOLUME 8 NUMBER 9

NUTRITION REVIEWS

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September 1950

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Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK, N. Y.

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NUTRITION REVIEWS

VOL. 8 SEPTEMBER, 1950 No. 9

CONCEPT OF GENETOTROPHIC DISEASE

A genetotrophic disease is one which occurs if a diet fails to provide a sufficient supply of one or more nutrients required at high levels because of the characteristic genetic pattern of the individual concerned (R. J. Williams, E. Beerstecher, and L. J. Berry, *Lancet* **I,** *287* (*1950*)). This concept based upon recent results in genetics and biochemistry is new in medical thought and is believed to be the basis for many diseases, the causation of which is now obscure. Individual patients are far from standardized specimens (R. J. Williams, "The Human Frontier," *Harcourt, New York* (*1946*)) and medical problems should consistently be considered in terms of the genetically diverse patients concerned rather than in terms of an abstract "normal."

Fundamental to the studies here outlined is the theory of partial genetic blocks (H. K. Mitchell and M. B. Houlahan, *Fed. Proc.* **6,** *506* (*1947*)). Such a block involves a heritable trait characterized by diminution, but not complete failure, of ability to carry out a specific enzymatic transformation, thereby increasing the need of the body for a specific nutritional factor or factors. The existence of partial blocks, for instance, may explain the difference between the dog and the rat in the efficiency of conversion of tryptophan into niacin (*Nutrition Reviews* **3,** *267* (*1945*); **7,** *83*, *307* (*1949*)).

The metabolic pattern of each person obviously includes characteristics that are individual and distinctive. Urinary and salivary analyses reveal these differences (R. C. Thompson and H. M. Kirby, *Arch. Biochem.* **21,** *210* (*1949*); R. J. Williams, L. J. Berry, and E. Beerstecher, *Ibid.* **23,** *275* (*1949*); *Science* **109,** *441* (*1949*)). Partial genetic blocks somewhere in the metabolic machinery are probably commonplace in individual inheritance. Without an augmented supply of the particular nutrient (or nutrients) which an individual needs because of his own peculiar genetic pattern, nutritional (genetotrophic) deficiency results.

Alcoholism was studied in its relation to individuality in metabolism. It was assumed in the beginning that the craving for alcohol has a physical and genetic basis similar to that of other special appetites that are under physiologic control. Experiments with rats and mice demonstrated, without serious doubt, that consumption of alcohol by laboratory animals is a genetotrophic phenomenon. Ordinary laboratory animals subsisting on stock diets, given a choice between 10 per cent alcohol and water, reacted variously as teetotalers, as moderate steady drinkers, as spasmodic drinkers, as drinkers with mounting appetites, etc. The drinking patterns appeared on a constant diet to be wholly under genetic control, but experiment demonstrated that they could be shifted by nutritional means from one extreme to the other. On marginal diets all of the rats eventually consumed large amounts of alcohol; on abundantly adequate diets none of the animals consumed more than a very small amount, even though a considerable appetite for alcohol had been developed previously. Different strains, highly inbred and otherwise, gave results in line with the genetic hypothesis. Unless a member of a highly inbred strain, each animal indicated possession of a distinctive metabolic pattern based presumably on its own partial genetic blocks and on consequent augmented requirements for specific vitamins, amino acids, or minerals without which deficiencies developed. The urge on the part of the animals to consume alcohol was conditioned by the existence of nutritional deficiencies.

Although the physiologic nature of appetite itself is unknown it is known that

alcohol has a sparing effect on thiamine (*Nutrition Reviews* **3,** *356* (*1945*)), that alcohol mobilizes vitamin A in the body (*Ibid.* **1,** *348* (*1943*)), and that there is increased urinary excretion of niacin metabolites and of thiamine if alcohol is substituted isocalorically in the diet (R. E. Butler and H. P. Sarett, *J. Nutrition* **35,** *539* (*1948*)). It is suggested that the basic etiologic factor in compulsive drinking is genetotrophic and that the social and psychologic factors only precipitate rather than cause the development of the clinical syndrome. Clinical trials on the treatment of compulsive drinkers are under way.

With these observations in mind and on the basis of the genetotrophic concept, the current pessimism in regard to genetic disease seems unwarranted.

The case for the genetotrophic approach to cancer is relatively strong. W. E. Heston (*Advances in Genetics* **II,** *99* (*1948*)) has reviewed some of the known hereditary factors in cancer, including data on spontaneous tumors in susceptible strains of mice. Extreme differences in the incidence of cancer in different racial groups have been noted (L. V. Ackerman and J. A. del Regato, "Cancer: Diagnosis, Treatment and Prognosis," *Mosby, St. Louis* (*1947*)), as well as high-familial incidence (C. H. Bateman, *Lancet* **I,** *500* (*1949*)). Although numerous attempts at nutritional therapy have been reported (J. P. Greenstein, "Biochemistry of Cancer," *Academic Press, Inc., New York* (*1947*)), and although no underlying nutritional defect has been discovered, there has never been a serious attempt to provide all nutritional requirements in quantities adequate to meet genetotrophic needs that may be several times above those considered normal.

Addition of "B vitamins" to a normal diet of rats has been shown to increase resistance to Jensen's rat sarcoma (G. M. Scott, *Lancet* **I,** *102* (*1949*)). A high-riboflavin content in rat liver tends to reduce the chance of development of tumors (E. Boyland, *Nature* **161,** *106* (*1948*)). Often, if not always, in a metabolically-active tumor there is a low level of "B vitamins" (M. A. Pollack, A. Taylor, and R. J. Williams, *Univ. Texas Pub.* **4237,** *56* (*1942*)), of riboflavin (J. Shack, *J. Nat. Cancer Inst.* **3,** *389* (*1943*)), of niacin (*Nutrition Reviews* **1,** *247* (*1943*)), and of vitamin B_6 (P. P. Cohen, *Cancer Research* **5,** *626* (*1945*)). Such evidence strongly suggests that nutritional and genetic factors may be combined in the problem of cancer. Even if the primary factors active in carcinogenesis are not genetotrophic, prophylactic treatment based on the genetotrophic concept may nevertheless be effective.

Diabetes mellitus possesses many known aspects suggesting a genetotrophic origin. Evidence for an hereditary basis is seen in the findings of H. Harris (*Proc. Roy. Soc. Med.* **42,** *326* (*1949*)) that older patients of the milder insulin-insensitive type are heterozygous for the abnormal gene, while younger patients are homozygous, that 30 to 50 per cent of all diabetics have a familial history of diabetes and that, if one uniovular twin has diabetes, the other invariably develops it (not true of binovular twins). Moreover, H. P. Himsworth (*Lancet* **I,** *465* (*1949*)) has indicated the widespread predisposition to hereditary or constitutional diabetes and the positive correlation of diabetic mortality (in patients over 45 years of age) with fat consumption. This does not mean necessarily that fat is deleterious, but that contingent variables may be important. Other similar considerations suggest the importance of nutrition in diabetes. It is noted that the beneficial effect of diet therapy in many cases requires an explanation beyond the present understanding of the diabetic syndrome.

Familial incidence of rheumatoid arthritis has been observed, although a genetic basis for the disease has not been established. The spectacular results obtained with cortisone indicate involvement of a specific biochemical lesion (*Nutrition Reviews* **7,** *290*

(*1949*)). The evidence for the importance of the "B vitamins" in sterol metabolism also suggests an impairment due to nutritional deficiency (M. L. Sweat and L. T. Samuels, *J. Biol. Chem.* **175,** 1 (*1948*)). As in other cases, the large number of earlier attempts at nutritional therapy are estimated to be entirely inadequate in light of the concept of the wide divergence of individual requirements.

The etiology of disseminated sclerosis has been shown to involve a genetic factor (D. Williams, *Proc. Roy. Soc. Med.* **39,** *599* (*1946*)). Multiple sclerosis resembles a biochemical or enzyme abnormality or, perhaps, a "toxi-defective process" (D. McAlpine, *Brain* **69,** *233* (*1946*)). Other studies have shown a history of subnormal health or malnutrition prior to the onset of the illness. Obesity seems to have a protective effect, and pregnancy, which is conducive to nutritional strain, is known to be a factor precipitating the disease. Demyelination, which is fundamental to disseminated sclerosis, has been duplicated experimentally by interference with oxidation (E. W. Hurst, *Ibid.* **67,** *103* (*1944*)). A vast amount of research on sclerosis is necessary before the genetotrophic concept of its origin can be disregarded.

The genetic basis for certain mental diseases, such as schizophrenia, has been recognized (F. J. Kallmann, *Am. J. Psychiat.* **103,** *309* (*1946*)) and an extensive literature has accumulated on the combined genetic and nutritional influences in many forms of mental disease. But nutritional therapy, while of acknowledged importance in the present treatment of nervous diseases, has been untried on the basis of the genetotrophic concept.

Further inquiry into mental symptoms associated with many vitamin deficiencies is needed. The effect of niacin on the dementia of pellagra is a dramatic and classic example of nutritional therapy. Numerous metabolic abnormalities in mental patients, generally regarded as psychogenic, need additional study in view of the intense metabolic activity of the central nervous system. It is pertinent that brain tissue clings tenaciously to its supply of vitamins (J. Salcedo, V. A. Najjar, L. E. Holt, and E. W. Hutzler, *J. Nutrition* **36,** *307* (*1948*)).

Darier's disease (E. Urbach and E. B. LeWinn, "Skin Diseases, Metabolism and Nutrition," *Grune and Stratton, New York* (*1946*)) is now generally believed to be due to a genetically induced high requirement for vitamin A.

The observations of N. C. Wetzel, W. C. Fargo, I. H. Smith, and J. Helikson (*Science* **110,** *651* (*1949*)) with respect to the response of certain malnourished children to the oral administration of vitamin B_{12}, are clearly in line with the genetotrophic concept as are the observations of T. L. Foster (*J. Nerv. Ment. Dis.* **110,** *1* (*1949*)) with respect to alleviation of "toxic psychosis" by administration of large amounts of niacin.

The genetotrophic concept can be extended to consideration of dental caries, allergies, anemias, sterility, habitual abortion, gout, and a host of geriatric problems. In the field of veterinary medicine, "wheat disease" (F. G. Harbaugh and J. Dennis, *Am. J. Vet. Res.* **8,** *396* (*1947*)) and "grass tetany" appear to be genetotrophic in character and to involve the existence in certain animals of unusually high requirements for calcium and magnesium, respectively.

The genetotrophic concept as outlined demands for its elucidation more information, particularly with respect to the magnitude of the gap which exists in the case of each nutrient, between the *average* requirement of the population generally, and the *maximum* requirement which any individual may have. Such data are now practically nonexistent. The importance of the genetotrophic concept hinges on such information. If these gaps are small, then the importance of the concept fades.

The recently developed insight into the tryptophan-niacin relationship suggests the possibility that a partial genetic block or

its equivalent might cause a metabolite hitherto not recognized as an essential nutrient, to become in a practical sense, a requirement for an affected individual.

It is not proposed that the diseases discussed are necessarily of purely genetotrophic origin. In every case genetotrophic factors are suspected to be at work. Partial enzymatic blocks, or their equivalent, probably occur in most instances of deranged metabolism and the site of these blocks may be determined by genetic factors. A block may be activated wholly by these genetic factors or may be occasioned by infections or by other processes. Partial genetic blocks constitute barriers between the nutritional elements and the metabolites essential for normal biologic functions.

Two ways of overcoming partial genetic blocks suggest themselves. The products of the blocked reaction may be supplied, but this is generally impracticable because of the complexity of the metabolic processes. A second and more reasonable procedure would be to increase the nutritional precursors to a concentration that would ensure necessary amounts of the metabolites despite an inefficient reaction. This augmentation of the precursors appears to be the practical answer to overcoming partial enzymatic blocks. The understanding of the rationale of this massive-type therapy is essential to the progress which we believe is imminent.

ROGER J. WILLIAMS
University of Texas
Austin, Texas

PRESENT KNOWLEDGE OF PTEROYLGLUTAMATES ("FOLIC ACID")

The intensive study of the chemistry, physiology, and clinical usefulness of the pteroylglutamates during the past two years makes it desirable to bring together the major developments since the writing of the last summary of the subject in this series (*Nutrition Reviews* **6,** *131 (1948)*). There exist a number of valuable detailed reviews of this family of compounds which will be useful to the reader desirous of greater detail than that included here (J. J. Pfiffner and A. G. Hogan, *Vitamins and Hormones* **4,** *1 (1946)*; W. J. Darby, *Ibid.* **5,** *119 (1947)*; B. L. Hutchings and J. H. Mowat, *Ibid.* **6,** *1 (1948)*; L. J. Berry and T. D. Spies, *Blood* **1,** *271 (1946)*; T. H. Jukes and E. L. R. Stokstad, *Physiol. Rev.* **28,** *51 (1948)*; J. F. Wilkinson, *Brit. Med. J.* **1,** *771, 822 (1948)*).

A series of papers from the Lederle Laboratories and Calco Chemical Division of American Cyanamid Company have presented the details of the isolation of the fermentation *Lactobacillus casei* factor as a product of the aerobic culture of an unidentified organism of the genus *Corynebacterium* (B. L. Hutchings *et al.*, *J. Am. Chem. Soc.* **70,** *1 (1948)*) as well as the isolation of the *L. casei* factor from liver (E. L. R. Stokstad, B. L. Hutchings, and Y. Subba-Row, *Ibid.* **70,** *3 (1948)*). The details of the degradative studies and the evidence leading to assignment of the accepted structure of the *L. casei* factor from liver (folic acid, pteroylglutamic acid, PGA) have been presented by workers from this same laboratory (Stokstad *et al.*, *Ibid.* **70,** *5 (1948)*; Hutchings *et al.*, *Ibid.* **70,** *10 (1948)*; J. H. Mowat *et al.*, *Ibid.* **70,** *14 (1948)*).

The liver *L. casei* factor (PGA) proved to be the simplest of the family of compounds. Inasmuch as it contains but one glutamic acid residue, it is often spoken of as the monoglutamate. Workers from the Cyanamid Company have described four methods of synthesis (C. W. Waller *et al.*, *J. Am. Chem. Soc.* **70,** *19 (1948)*; M. E. Hultquist *et al.*, *Ibid.* **70,** *23 (1948)*; R. B. Angier *et al.*, *Ibid.* **70,** *25 (1948)*; J. H. Boothe *et al.*, *Ibid.* **70,** *27 (1948)*).

The fermentation factor has proved to be

a similar pteroyl derivative containing three glutamic acid residues in the side chain. The structure indicates that these glutamic acid residues could be linked through either alpha- or beta-peptide bonds. Neither synthetic pteroyl-alpha-glutamyl-glutamic acid nor pteroyl-alpha-gamma-glutamyl-diglutamic acid was identical with the fermentation factor (J. H. Mowat *et al.*, *J. Am. Chem. Soc.* **70,** *1096 (1948)*). J. H. Boothe *et al.* (*Ibid.* **70,** *1099 (1948)*) synthesized pteroyl-gamma-glutamyl-gamma-glutamyl-glutamic acid. Although the compound was not isolated, it exhibited microbiologic activity similar to the naturally-occurring fermentation factor. Accordingly, it seems certain that the three glutamic acid residues of the fermentation factor triglutamate are linked together through gamma-peptide bonds as is the glutamic peptide, glutathione. Further, the glutamic acid in these compounds appears to be the naturally-occurring L-glutamic acid.

The conjugate present in yeast is the heptaglutamate (J. J. Pfiffner, D. G. Calkins, E. S. Bloom, and B. L. O'Dell, *J. Am. Chem. Soc.* **68,** *1392 (1946)*), presumably again a gamma peptide. This exact linkage, however, does not appear to have been established. The synthetic conjugate has not been prepared. A synthetic gamma-linked dipeptide (Mowat *et al.*, *loc. cit.*) has biologic activity as a folic acid but is not known to occur in nature.

E. P. Daniel and O. L. Kline (*J. Biol. Chem.* **170,** *739 (1947)*) define the stability of folic acid during extraction, sterilization, and storage involved in microbiologic assays. Destruction is most rapid during autoclaving in acid solution. In addition to the several microbiologic assays, folic acid may be determined colorimetrically by coupling the diazotized reduction product with the Bratton-Marshall reagent (B. L. Hutchings *et al.*, *Ibid.* **168,** *705 (1947)*; A. J. Glazko and L. M. Wolf, *Arch. Biochem.* **21,** *241 (1949)*), fluorometrically after oxidation with permanganate (V. Allfrey, L. J. Teply, C. Geffen, and C. G. King, *J. Biol. Chem.* **178,** *465 (1949)*) or by its effect on the pupation of the larva of the mosquito, *Aëdes aegypti L* (L. Golberg, B. De Meillon, and J. F. Murray, *Nature* **160,** *22 (1947)*). Exposure to sunlight, but not to fluorescent light, inactivates pteroylglutamic acid (E. L. R. Stokstad, D. Fordham, and A. de Grunigen, *J. Biol. Chem.* **167,** *877 (1947)*), hence solutions should be protected from strong irradiation.

Knowledge of the distribution of the pteroylglutamates in foodstuffs remains incomplete and is complicated by lack of information concerning the relative activities for man of the three possible naturally-occurring pteroylglutamates (folic acid, the fermentation factor, the heptaglutamate). Fresh green leafy vegetables and organ meats are rich sources. Lean beef and veal and wheat cereals appear to be intermediate in content. Root vegetables, light green leafy vegetables, canned foods, dairy foods, and pork seem to be low in the vitamin (O. E. Olson, R. H. Burris, and C. A. Elvehjem, *J. Am. Dietet. Assn.* **23,** *200 (1947)*). Assays of feedstuffs, employing chicks, reveal that yeast and liver meals are excellent sources and that soybean meal, alfalfa meal, and fermentation solubles are good sources of the vitamin (R. J. Lillie and G. M. Briggs, *Poultry Sci.* **26,** *289 (1947)*). No difference existed in the folic acid content of normal as contrasted to diseased human hearts (E. S. Pallares, H. M. Garza, H. Aguirre, and I. D. Urdanivia, *Arch. Biochem.* **22,** *31 (1949)*).

A great variety of pterins structurally related to folic acid have been studied for their inhibitory effects in microorganisms as well as in animals (L. J. Daniel and L. C. Norris, *J. Biol. Chem.* **170,** *747 (1947)*; Daniel, Norris, M. L. Scott, and G. F. Heuser, *Ibid.* **169,** *689 (1947)*; **173,** *123 (1948)*; M. E. Swendseid *et al.*, *Ibid.* **179,** *1175 (1949)*; D. W. Woolley and A. Pringle, *Ibid.* **174,** *327 (1948)*). In addition to methylfolic and pteroylaspartic acid previously discussed (*Nutrition Reviews* **6,** *131 (1948)*) a

new analogue with potent antagonistic effect in rats, chicks, chick embryos, guinea pigs, and other species has been developed. This analogue is 4-amino-10-methyl-pteroylglutamic acid (A. L. Franklin, M. Belt, E. L. R. Stokstad, and T. H. Jukes, *J. Biol. Chem.* **177,** *621* (*1949*); F. S. Philips and J. B. Thiersch, *J. Pharmacol. Exp. Therap.* **95,** *303* (*1949*); D. A. Karnofsky, P. A. Patterson, and L. P. Ridgway, *Proc. Soc. Exp. Biol. Med.* **71,** *447* (*1949*); J. Innes, E. M. Innes, and C. V. Moore, *J. Lab. Clin. Med.* **34,** *883* (*1949*); P. F. Wagley and H. R. Morgan, *Arch. Path.* **46,** *441* (*1948*)). Great impetus has been given to the study of these antagonists by the demonstration that some may inhibit the growth of implanted and spontaneous tumors in experimental animals (*Nutrition Reviews* **7,** *82* (*1949*); R. Lewisohn *et al.*, "Approaches to Tumor Chemotherapy," *p. 139, Am. Assn. Advancement Sci.* (*1947*); C. W. Waller *et al.*, *Tr. N. Y. Acad. Sci.* **10,** *68* (*1948*); E. B. Schoenbach, A. Goldin, B. Goldberg, and L. G. Ortega, *Cancer* **2,** *57* (*1949*)). The severity of transplanted leukemia in mice can be partially controlled by administration of a folic antagonist (D. R. Weir, A. D. Welch, and R. W. Heinle, *Proc. Soc. Exp. Biol. Med.* **71,** *107* (*1949*)). Various folic acid antagonists have been employed therapeutically in human leukemia. In this disease, temporary remissions are induced in approximately one-third of the cases but cure of the disease has not been accomplished (W. Dameshek, *Blood* **4,** *168* (*1949*); S. Farber, *Ibid.* **4,** *160* (*1949*)). Further, there is some evidence that pteroyltriglutamate (fermentation factor, not an antagonist) may afford some symptomatic relief in patients with advanced malignancies (S. M. Hardy, A. L. Joyner, and R. W. Howard, *N. Y. State J. Med.* **49,** *2697* (*1949*)). "Toxic" manifestations of the antagonists consist of glossitis, diarrhea, gastrointestinal lesions, and anemia—in effect the syndrome of folic acid deficiency.

An especially interesting series of compounds, some of which have slight folic acid-like activity, others of which exhibit antagonism to folic acid are certain derivatives of benzimidazole, structurally related to the pteroylglutamates (P. C. Edwards, D. Starling, A. M. Mattocks, and H. E. Skipper, *Science* **107,** *119* (*1948*)). A particular interest in these compounds resides in the fact that the newer hematopoietic vitamin B_{12} has been shown to contain a dimethylbenzimidazole grouping (K. Folkers, *Chem. Eng. News* **28,** *1634* (*1950*)).

The apparent antagonism of sulfonamides for the pteroylglutamates would seem to reside in the interference by the sulfonamide in the synthesis of pteroylglutamic acid through competition with para-aminobenzoic acid, a moiety which is part of the folic acid molecule (J. O. Lampen and M. J. Jones, *J. Biol. Chem.* **166,** *435* (*1946*); R. H. Nimmo-Smith, J. Lascelles, and D. D. Woods, *Brit. J. Exp. Path.* **29,** *264* (*1948*)). The addition of a sulfonamide, succinylsulfathiazole, to the diet of the rabbit reduces the urinary excretion of folic acid (R. E. Simpson, B. S. Schweigert, and P. B. Pearson, *Proc. Soc. Exp. Biol. Med.* **70,** *611* (*1949*)). Such findings are consistent with the interpretation that the rabbit normally derives a considerable quantity of folic acid from the microorganisms of the gastrointestinal tract and that in the presence of sulfonamides this synthesis by the flora is impeded.

Conjugated forms of folic acid are converted into the monoglutamic acid (PGA) by the action of specific tissue enzymes known as conjugases. In the healthy adult, intravenously administered pteroyltriglutamate is converted rapidly into PGA (B. S. Schweigert, *J. Lab. Clin. Med.* **33,** *1271* (*1948*)). An absence or decrease in quantity of tissue conjugases is no longer held to explain the defect in pernicious anemia, but additional investigations of the conjugate-conjugase relationship in healthy individuals and in pernicious anemia and related syndromes are warranted.

Accompanying conjugase in several natural products is an inhibitor known as conjugase inhibitor. This conjugase inhibitor seems to influence the availability of the conjugated folic acid to healthy human subjects. Oral administration of the heptaglutamate (folic acid conjugate) alone (no accompanying inhibitor) was followed by urinary excretion levels of pteroylglutamic acid similar to those obtained after administration of the free vitamin (PGA). When the orally administered heptaglutamate was accompanied by conjugase inhibitor, lower excretion levels resulted (M. E. Swendseid, O. D. Bird, R. A. Brown, and F. H. Bethell, *J. Lab. Clin. Med.* **32,** *23* (*1947*)). It is apparent, therefore, that estimations of folic acid available from foodstuffs will involve both the determination of the quantities of the different forms of folic acid present and of the inhibitors that may exist in the foodstuffs.

An exact metabolic role cannot as yet be assigned to folic acid. Nevertheless, many promising leads on the problem have appeared. It seems reasonable that folic acid, like other members of the B-complex, enters into combination with a specific protein and thereby functions as an enzyme. Indeed V. G. Allfrey and C. G. King (*J. Biol. Chem.* **182,** *367* (*1950*)) have presented evidence for the existence in yeast of a specific folic acid-protein complex containing approximately 160 micrograms of folic acid as the heptaglutamate per gram of protein. This folic acid-protein complex has not been identified with a known enzymic effect. W. H. Prusoff, L. J. Teply, and King (*Ibid.* **176,** *1309* (*1948*)) have shown that a partial deficiency of PGA resulted in a decrease in desoxyribonucleic acid content of *L. casei*. This effect was not caused by a deficiency of other factors. Similarly, the desoxypentosenucleic acid content of livers of chicks deficient in PGA was somewhat lower than that of corresponding birds which had received the vitamin (J. R. Totter *et al.*, *Proc. Soc. Exp. Biol. Med.* **70,** *435* (*1949*)). This latter difference, however, was not striking and is of doubtful significance.

The observation by C. K. Keith *et al.*, (*J. Biol. Chem.* **176,** *1095* (*1948*)) that there is an inverse relationship between the PGA content of the diet and the liver xanthine oxidase of chicks has been confirmed (J. N. Williams, C. A. Nichol, and C. A. Elvehjem, *Ibid.* **180,** *689* (*1949*)). H. M. Kalckar and H. Klenow (*Ibid.* **172,** *349* (*1948*)) found that xanthine oxidase was inhibited *in vitro* by PGA. Later, it was recognized that this inhibition was not exhibited by a highly purified sample of PGA and the inhibition of this oxidase and similar enzymes has been attributed to the impurity, 6-pteridyl aldehyde (Kalckar, N. O. Kjeldgaard, and Klenow, *Ibid.* **174,** *771* (*1948*)). Studies of the inhibitory effects of this compound support the view that xanthine oxidase and xanthopterin oxidase of cream are identical enzymes (O. H. Lowry, O. A. Bessey, and E. J. Crawford, *Ibid.* **180,** *389, 399* (*1949*)). J. N. Williams and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **71,** *303* (*1949*)), however, have found that both commercial and aldehyde-free folic acid inhibit the oxidation of xanthine by purified preparation of xanthine oxidase. It is of interest that this 6-formyl pteridine exerts an inhibitory effect on oxygen uptake of rat-brain suspension in the presence of pyruvate, an effect which is not shared by pteroylglutamic acid free of the impurity (S. Cobb, O. H. Pearson, and A. B. Hastings, *Ibid.* **70,** *595* (*1949*)).

This formyl degradation product of folic acid which has inhibitory effects for a number of enzymes is not to be confused with the substance referred to as formylfolic acid, a formamido derivative of folic acid in which the aldehyde grouping is attached to the nitrogen of the para-aminobenzoic acid moiety. Formylfolic is analogous in structure to rhizopterin. It has been suggested that it may serve as a carrier of formate (M. Gordon, J. M. Ravel, R. E. Eakin, and W. Shive, *J. Am. Chem. Soc.* **70,** *878* (*1948*)). It has been suggested that formylfolic may

function to incorporate a single carbon unit into an imidazole precursor of purines (*Nutrition Reviews* **6,** *211* (*1948*); W. Shive *et al.*, *J. Am. Chem. Soc.* **69,** *725* (*1947*)). Many microbiologic studies indicate a possible role of PGA in the biosynthesis of thymine.

Folic acid has been related to the metabolism of tyrosine. An increased excretion in the urine of phenolic compounds (derived from tyrosine) occurs in pernicious anemia and this excretion level is decreased by administration of liver extract or desiccated stomach preparations (*Nutrition Reviews* **6,** *70* (*1948*)). Human infants and guinea pigs show abnormal excretion of metabolites of the aromatic amino acids (phenylalanine and tyrosine) when placed on diets deficient in ascorbic acid and high in these amino acids. This condition is relieved by dosing with ascorbic acid (*Ibid.* **5,** *173* (*1947*)). If pteroylglutamic acid is administered to scorbutic guinea pigs ingesting added quantities of tyrosine the abnormal excretion of metabolites is prevented. Further, the hydroxyphenyluria once established in the scorbutic tyrosine-fed guinea pig can be erased by large doses of parenterally administered PGA (C. W. Woodruff, M. E. Cherrington, A. K. Stockell, and W. J. Darby, *J. Biol. Chem.* **178,** *861* (*1949*)). PGA seems, therefore, to have some role in tyrosine metabolism and possibly to be related to ascorbic acid through this mechanism. C. D. Govan and H. H. Gordon (*Science* **109,** *332* (*1949*)) have found an inconstant effect of PGA upon the hydroxyphenyluria of premature infants. In infants, with scurvy, who were fed additional quantities of tyrosine, C. W. Woodruff (*personal communication*) found no effect upon the hydroxyphenyluria when PGA was administered in doses of 15 mg. per day. When overwhelming doses of 90 mg. of PGA daily were given to scorbutic infants the expected tyrosyluria was inhibited (J. E. Morris, E. R. Harpur, and A. Goldbloom, *J. Clin. Invest.* **29,** *325* (*1950*)). Other evidence for a relationship between tyrosine metabolism and folic acid includes the observation that some antifolic compounds inhibit dopa decarboxylase (G. J. Martin and J. M. Beiler, *J. Am. Pharm. Assn.* **37,** *32* (*1948*)). It is possible that the abnormalities of feather pigmentation which have been observed in folic acid-deficient chicks (R. J. Lillie and G. M. Briggs, *Poultry Sci.* **26,** *475* (*1947*)) are a manifestation of aberrant tyrosine metabolism.

In addition to the similar effects of folic acid and vitamin C upon tyrosine metabolism, there are other indications of a relationship between the two vitamins. Several of these have been outlined by Woodruff *et al.* (*loc. cit.*) and include the inconstant hematopoietic effect of ascorbic acid in adult scurvy and the clinical association of megaloblastic anemia of infancy with diets low in vitamin C. Vitamin C influences the leukopenia of the PGA-deficient rat (B. C. Johnson and A. S. Dana, *Science* **108,** *210* (*1948*)) and permits greater growth of the chick with a semipurified diet devoid or containing low levels of folic acid (L. S. Dietrich, C. A. Nichol, W. J. Monson, and C. A. Elvehjem, *J. Biol. Chem.* **181,** *915* (*1949*)). These latter workers have indicated that vitamins C and B_{12} stimulate the synthesis of folic acid *in vivo* as revealed by increased stores in the liver.

C. D. May and co-workers (*Bull. Univ. Minn. Hosp.* **21,** *208* (*1950*)) have produced additional important evidence bearing on this relationship. These workers have found that the folic acid-deficient monkey fails to become anemic if he is supplied with sufficient quantities of ascorbic acid. Having once become anemic on a low-folic-low-ascorbic acid regimen, however, a hematopoietic response is produced only with folic acid.

Miscellaneous effects attributed to folic acid include some role in blood glucose regulation in the chick, inasmuch as folic acid-deficient birds exhibited a relative hyperglycemia (J. N. Williams, C. A. Nichol, and C. A. Elvehjem, *J. Biol. Chem.* **180,** *689* (*1949*)). Fibrinolysin, a proteolytic enzyme potentially present in plasma with the

ability to decompose fibrinogen and fibrin, is inactivated by a substance termed "antifibrinolysin." Antifibrinolysin is present in the plasma of all species. In the chick maintained on a PGA-deficient diet, there occurs an increase in the antifibrinolysin activity in the plasma. This increase is prevented by administration of crystalline PGA (M. M. Guest, A. G. Ware, and W. H. Seegers, *Am. J. Physiol.* **150,** *661* (*1947*)). PGA increases the fecal porphyrin excretion of rats fed a purified diet. It decreases the growth inhibition of rats brought about by feeding 3 per cent sodium benzoate (J. R. Totter, E. S. Amos, and C. K. Keith, *J. Biol. Chem.* **178,** *847* (*1949*)). When folic acid was administered to monkeys rendered deficient by combination of a folic acid-deficient diet plus aminopterin, the recovery phase was marked by an increased urinary output of uric acid, allantoin, and a very high creatinuria. It was suggested that folic acid is involved with creatine formation (J. S. Dinning and P. L. Day, *Ibid.* **181,** *897* (*1949*)).

Folic acid is important in the reproductive processes. This is indicated by the impaired reproductive performance of the rat which results from a low intake of PGA during pregnancy (M. M. Nelson and H. M. Evans, *J. Nutrition* **38,** *11* (*1949*)) and the increased requirement for folic acid during lactation (L. R. Cerecedo and L. Mirone, *Arch. Biochem.* **12,** *154* (*1947*); Nelson and Evans, *Ibid.* **13,** *265* (*1947*); M. B. Williamson, *Proc. Soc. Exp. Biol. Med.* **70,** *336* (*1949*)). Further, it has been shown that the characteristic tissue response to estrogen administration by both the monkey and chick is dependent upon an adequate supply of folic acid (*Nutrition Reviews* **6,** *284* (*1948*)). R. Hertz (*Science* **107,** *300* (*1948*)) has now demonstrated that a folic acid antagonist interferes with tissue growth response to estrogen administration in the chick.

Folic antagonists bring about definite changes in the hematopoietic tissue of embryonic eggs (P. F. Wagley and H. R. Morgan, *Arch. Path.* **46,** *441* (*1948*)) and produce abnormalities of development of the embryo when injected into the incubating egg of the chick (*Nutrition Reviews* **8,** *42* (*1950*)). A deficiency of the vitamin in the maternal diet produces a low incidence of hydrocephalus in young rats (B. L. O'Dell, J. R. Whitley, and A. G. Hogan, *Proc. Soc. Exp. Biol. Med.* **69,** *272* (*1948*)).

The early observations of E. E. Snell and H. K. Mitchell (*Proc. Nat. Acad. Sci.* **27,** *1* (*1941*)) and E. L. R. Stokstad (*J. Biol. Chem.* **139,** *475* (*1941*)) indicated that thymine and purines are growth promoting for *Streptococcus faecalis* and *L. casei* in the absence of sources of folic acid. J. L. Stokes (*J. Bact.* **48,** *201* (*1944*)) could not demonstrate folic acid in organisms grown under conditions of folic acid deprivation plus thymine. The hypothesis was set forth that folic acid serves as a coenzyme for the synthesis of thymine or related compounds—an interpretation which has been both confirmed (L. L. Rogers and W. Shive, *J. Biol. Chem.* **172,** *751* (*1948*)) and denied (H. G. Petering and R. A. Delor, *Science* **105,** *547* (*1947*)). At any rate, in man, thymine possesses hematopoietic activity qualitatively similar to folic acid (T. D. Spies, W. B. Frommeyer, C. F. Vilter, and A. English, *Blood* **1,** *185* (*1946*)). The dosage requirement is much higher than that of folic acid, and thymine does not have anticombined system disease activity. Thymine does not substitute for folic acid in the rat (Petering and Delor, *Science* **110,** *185* (*1949*)) or chick (Petering, *Nutrition Reviews* **6,** *255* (*1948*)). The final relationship between these substances remains to be determined. It is important, however, to recognize that there are substances without folic acid activity for some species which possess activity for others, and that a metabolic relationship that seems logical for one species may not necessarily be generalized to others.

Folic acid seems somehow interrelated with choline in higher organisms. In both chicks and rats it has a choline-sparing effect.

PGA, however, cannot replace choline completely (*Nutrition Reviews* **8,** *145* (*1950*)). It seems to aid in the utilization of homocystine by the rat (M. A. Bennett, *Science* **110,** *589* (*1949*)). The question may be raised whether this implies that folic acid may play a role in the formation of methyl groups. Choline has been reported to exert some hematopoietic activity in certain anemias in man (*Nutrition Reviews* **6,** *89* (*1948*)). On the other hand, it has been reported that choline or acetylcholine administration may result in production of a "hyperchromic anemia" which can be alleviated by folic acid (J. E. Davis, *Am. J. Physiol.* **147,** *404* (*1946*)). M. F. Clarkson and C. H. Best (*Science* **105,** *622* (*1947*)) have failed to produce an anemia in dogs by choline feeding.

In addition, Davis (*loc. cit.*) reported that serum cholinesterase was increased in human subjects following administration of folic acid. This worker has also reported the occurrence of increased acetylcholine in the serum of patients with pernicious anemia and the decrease in this substance following therapy with folic acid or other active hematopoietic agents (J. E. Davis, *Science* **104,** *605* (*1946*)). This is in keeping with the finding that patients with pernicious anemia in relapse exhibit an abnormally low cholinesterase content of their blood and that the level is raised to normal by folic acid administration (L. M. Meyer, A. Sawitsky, N. D. Ritz, and H. M. Finch, *J. Lab. Clin. Med.* **33,** *189* (*1948*)). In view of the failure of folic acid to protect the nervous system of the patient with pernicious anemia, it is of interest that Davis and D. E. Fletcher (*J. Pharmacol. Exp. Therap.* **88,** *246* (*1946*)) have observed multiple hemorrhages into the grey matter of the brain and spinal cord and diffuse gliosis and acute neurone changes in anemic dogs receiving choline. The exact relationship of these factors is obscure, but the subject is worthy of clarification.

An increasing body of evidence indicates that there may exist precursors as well as more active metabolites of the pteroylglutamates. Prior to the identification of the pteroylglutamates, there was evidence for the existence of potential precursors of folic acid and that xanthopterin when incubated with rat or monkey liver plus yeast greatly increased the folic acid activity above that of liver plus yeast alone (J. R. Totter, V. Mims, and P. L. Day, *Science* **100,** *223* (*1944*)). This activity of liver seemed to be explained by the liberation of conjugated folic acid by conjugase of the tissues. O. E. Olson, E. E. C. Fager, R. H. Burris, and C. A. Elvehjem (*J. Biol. Chem.* **174,** *319* (*1948*)) now question that this explanation suffices, since it appears that a dialyzable, heat-stable precursor of folic acid is liberated (activated) from liver under conditions at which conjugase is not active. A similar finding is reported by D. A. Hall (*Nature* **162,** *178* (*1948*)) who has suggested that the precursor may be an unstable hexone base produced during the oxidative deamination of histidine.

A substance which may be a metabolite of folic acid and is required by the microorganism *Leuconostoc citrovorum* has been termed the citrovorum factor (H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.* **176,** *165* (*1948*); *Nutrition Reviews* **8,** *282* (*1950*)). This factor is present in liver preparations designed for treating pernicious anemia and yet it is differentiated from vitamin B_{12} and folic acid. On a weight basis it appears to be much more active than the latter vitamin. The content of the citrovorum factor in human or in rat urine is greatly enhanced by administering folic acid (Sauberlich, *J. Biol. Chem.* **181,** *467* (*1949*)). It appears that the "folinic acid" proposed by Shive is a citrovorum factor (T. J. Bardos, T. J. Bond, J. Humphreys, and W. Shive, *J. Am. Chem. Soc.* **71,** *3852* (*1949*)). Further study of the citrovorum factor in animals and in man may be expected to yield valuable facts.

Although there have appeared many clinical reports of the use of folic acid in the treatment of various anemias in man, few new concepts seem to have arisen since the

previous summary. Folic acid is active hematopoietically in the treatment of pernicious anemia, but it does not protect against the development of combined system disease (neurologic complication of pernicious anemia). The unfortunate implication (Editorial, *New Engl. J. Med.* **237,** *713 (1947)*; J. F. Ross, H. Belding, and B. L. Paegel, *Blood* **3,** *68 (1948)*) that folic acid acts as a metabolic antagonist in the metabolism of glutamic acid has not been substantiated by careful study (A. L. Franklin *et al.*, *Proc. Soc. Exp. Biol. Med.* **67,** *523 (1948)*). Careful evaluation of the therapeutic place of folic acid by W. Dameshek (*Blood* **3,** *699 (1948)*) pointed to the usefulness of the vitamin and indicated the permanent place of folic acid in therapy. While the vitamin will not protect the nervous system of patients with pernicious anemia, it does not exert a "toxic action" which will cause neurologic disease (J. Weissberg, T. H. McGavack, M. Vogel, and S. Kenigsberg, *Ibid.* **5,** *148 (1950)*).

Folic acid is therapeutically effective in reversible sprue, in nutritional macrocytic anemia, in megaloblastic anemia of infancy, and in "pernicious anemia of pregnancy." Vitamin B_{12} is not effective in the latter disease (F. H. Bethell, M. C. Meyers, and R. B. Neligh, *J. Lab. Clin. Med.* **33,** *1477 (1948)*; L. A. Day, B. E. Hall, and G. L. Pease, *Proc. Staff Meetings Mayo Clinic* **24,** *149 (1949)*; R. H. Furman *et al.*, *Am. Practitioner Digest Treat.* **1,** *146 (1950)*), while it is but sometimes effective in the anemia of infancy (C. W. Woodruff, H. W. Ripy, J. C. Peterson, and W. J. Darby, *Pediatrics* **4,** *723 (1949)*). Folic acid appears to be consistently effective in both these latter anemias.

The toxicology of massive doses of folic acid is being studied. The major damage resulting from massive doses appears in the kidney. B. K. Harned, R. W. Cunningham, H. D. Smith, and M. C. Clark (*Ann. N. Y. Acad. Sci.* **48,** *289 (1946)*) noted tubular blockage with material judged to be precipitated PGA. Similar renal tubular casts in association with weight loss have been noted in guinea pigs dosed with 1 to 5 mg. of PGA daily (J. R. Dawson, C. W. Woodruff, and W. J. Darby, *Proc. Soc. Exp. Biol. Med.* **73,** *646 (1950)*). In mice, injections of PGA have produced a decrease in renal alkaline phosphatase activity and an increase in weight of the kidney (G. G. Villela and M. I. Mello, *Ibid.* **70,** *453 (1949)*). Of unusual interest is the finding that the lethal dose of PGA is considerably greater for male mice than for females (A. Taylor and N. Carmichael, *Ibid.* **71,** *544 (1949)*).

This incomplete review serves to indicate the major developments related to the pteroylglutamates. It is apparent that with the accumulation of so much information at the fundamental level we can look forward to a clearer understanding of the exact role of this vitamin in metabolism and in disease.

RATE OF PLASMA PROTEIN PRODUCTION AND DEGRADATION

The plasma proteins are maintained at a remarkably constant concentration in normal individuals, presumably due to an equal rate of production and destruction. In many disease states the plasma protein concentration is reduced. This is particularly true in liver disease and idiopathic hypoproteinemia. Since the liver is the chief site of formation of some of the plasma proteins (albumin, fibrinogen, prothrombin) it has been assumed that in diseases of this organ the low-plasma protein concentration (chiefly albumin) is related to a diminished rate of formation, while the rate of destruction is maintained at a normal level. The relatively slow availability, but nutritional adequacy, of intravenously injected plasma proteins, including albumin, has been noted previously (*Nutrition Reviews* **6,** *152 (1948)*; F. Albright, A. P. Forbes, E. C. Reifen-

stein, *Tr. Assn. Am. Phys.* **59,** *221* (*1946*); C. Waterhouse, S. H. Bassett, and J. W. Holler, *J. Clin. Invest.* **28,** *245* (*1949*)). In fact some workers have observed that albumin so administered has a 50 per cent disappearance time of roughly five days.

W. Parson *et al.* (*J. Clin. Invest.* **29,** *381* (*1950*)) in a metabolic study of 2 patients with infectious hepatitis have shown that in this disease, too, repeated intravenous injections of albumin produce a rise in albumin concentration, followed by a slow fall after administration is stopped. Urine nitrogen did not increase so that most, if not all, of the albumin nitrogen was retained to form body tissue. That the conversion of albumin to body tissue protein was delayed is indicated not only by the rise in plasma albumin concentration, but also by a delay in phosphorus retention. Thus during the period of albumin infusions positive nitrogen balance was accompanied by a rising plasma albumin concentration, but no increase in phosphorus retention, indicating retention in the body of albumin as such. After the infusions were stopped the plasma albumin concentration tended to decline and phosphorus was retained, indicating utilization of the albumin nitrogen for body tissue protein production.

L. W. Kinsell *et al.* (*J. Clin. Invest.* **29,** *238* (*1950*)) have used methionine labeled with radioactive sulfur given intravenously as a tracer for studying the rate of plasma protein synthesis and degradation in normal individuals, 2 patients with chronic liver disease, one with idiopathic hypoproteinemia, and one with Cushing's syndrome.

Following administration of methionine labeled with radioactive sulfur, there was a rapid incorporation of this material into plasma protein as measured at suitable intervals by the specific activity of the total plasma protein. The maximum activity was reached in approximately eight hours in the normal controls and in the patients studied. Following this there was a plateau of specific activity until the end of twenty-four hours. Thereafter a gradual decline in specific activity occurred indicating, the authors believed, a gradual degradation or catabolism of the plasma protein.

The rate of incorporation of labeled methionine into the plasma protein of these individuals was significantly less in the 3 patients with chronic liver disease than in the normals and greatly increased in the patient with idiopathic hypoproteinemia in whom the study was done twice, and in the patient with Cushing's syndrome. Thus the authors conclude that relative to the normal individuals, those with chronic liver damage had a diminished rate of plasma protein formation, and the patient with Cushing's syndrome and the one with idiopathic hypoproteinemia had a markedly increased rate of protein synthesis.

The rate of disappearance of plasma protein as measured by the reduction in specific activity was approximately the same for the patients with chronic liver damage as for the normal individuals, but the patient with idiopathic hypoproteinemia in both studies had a very much more rapid rate of disappearance of labeled methionine after the first twenty-four-hour period. The authors interpret this as indicating a much more rapid rate of catabolism of plasma protein in idiopathic hypoproteinemia than in normal individuals, but that the catabolism in chronic liver disease is about the same as in the normal. Using other metabolic methods, F. Albright, F. C. Bartter, and A. P. Forbes (*Tr. Assn. Am. Phys.* **62,** *204* (*1949*)) reached the same conclusion in a patient with idiopathic hypoproteinemia.

The urinary excretion of total labeled sulfate was used by the authors as an index of catabolism of the labeled methionine which had been administered. Approximately half of the original labeled sulfur was still present in the normal individual at the end of five weeks. Presumably part of this was in plasma protein and part in other body protein. In the patients with liver disease an increased sulfate excretion occurred during the first

forty-eight hours and was attributed to catabolism of the isotopic methionine in excess of that converted into protoplasm. In the patient with idiopathic hypoproteinemia the increased rate of excretion of urinary sulfur was well above normal and progressive after the first twenty-four hours, indicating, the authors suspect, a hypercatabolism.

These data suggest that in a normal individual proteins are being built and destroyed at a relatively constant rate, that the degradation of plasma protein is a relatively slow process, that there is a defect in protein anabolism in liver disease, while in idiopathic hypoproteinemia an excessive rate of plasma protein catabolism occurs.

BIOCHEMISTRY OF THE METHYL GROUP

Despite the structural simplicity of the methyl group, its elaboration in certain chemical linkages may be possible only to a limited extent in the higher animals. The ability of such methyl groups to be transferred intact from certain linkages to others has been recognized for over a decade. Recent findings have the effect of preventing the overextension of the concept of transmethylation in three respects:

1. Methyl donors have been shown not to be equivalent, and methyl transfers not reversible in a precise sense. The concept of a "methyl pool" of equivalent methyl donors appears to be of questionable validity.

2. "Labile" methyl groups have been shown to be subject to active degradation.

3. Some ability of the animal organism to synthesize "labile" methyl groups is indicated.

It has been shown rather clearly that choline is not a direct methylating agent in the sense of a reversal of its formation by the methylation of aminoethanol derivatives. Instead, choline apparently donates a single labile methyl group only after being oxidized to betaine. J. W. Dubnoff (*Fed. Proc.* **8,** *195 (1949)*) showed that choline and homocysteine together did not lead to methionine formation except in animals possessing choline oxidase. Homogenates of livers of species not possessing such an enzyme (the rabbit, the chick, and the guinea pig) formed methionine from betaine, dimethylthetin, and dimethylpropiothetin both anaerobically and aerobically. These compounds may be represented by the structure $(CH_3)_2S^+(CH_2)_nCOOH$, where n = 1 or 2. Dubnoff and H. Borsook (*J. Biol. Chem.* **176,** *789 (1948)*) partially purified an enzyme which transfers a methyl group from dimethylthetin, or from dimethylpropiothetin, to homocysteine. Dimethylthetin was ten to twenty times as effective a methyl donor as betaine in tissue homogenates. The transmethylases for these two substances were not the same enzyme, but were distinguishable by a difference in stability at pH 4.

J. A. Muntz (*J. Biol. Chem.* **182,** *489 (1950)*) synthesized choline containing an excess of N^{15}. This was allowed to react with homocysteine in a rat-liver homogenate, and unlabeled dimethylaminoethanol and dimethylglycine were added as carriers. If choline donated a methyl group in the sense of a reversal of its formation, dimethylaminoethanol should be a product. Reisolation of the dimethylaminoethanol, however, recovered none of the labeled nitrogen. On the other hand, the reisolated dimethylglycine contained large excesses of the isotope. This is the product to be expected if betaine donates a single methyl group. The anaerobic conditions observed would be expected to show some nonoxidative methyl donation if choline were subject to such a reaction.

G. L. Cantoni (*Fed. Proc.* **9,** *158 (1950)*) has studied the transfer of a methyl group from methionine to nicotinamide by liver extract. Betaine was an active donor only in the presence of homocysteine, indicating presumably that the transfer occurred by

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3. Some ability of the animal organism to synthesize "labile" methyl groups is indicated.

It has been shown rather clearly that choline is not a direct methylating agent in the sense of a reversal of its formation by the methylation of aminoethanol derivatives. Instead, choline apparently donates a single labile methyl group only after being oxidized to betaine. J. W. Dubnoff (*Fed. Proc.* **8,** *195 (1949)*) showed that choline and homocysteine together did not lead to methionine formation except in animals possessing choline oxidase. Homogenates of livers of species not possessing such an enzyme (the rabbit, the chick, and the guinea pig) formed methionine from betaine, dimethylthetin, and dimethylpropiothetin both anaerobically and aerobically. These compounds may be represented by the structure $(CH_3)_2S^+(CH_2)_nCOOH$, where n = 1 or 2. Dubnoff and H. Borsook (*J. Biol. Chem.* **176,** *789 (1948)*) partially purified an enzyme which transfers a methyl group from dimethylthetin, or from dimethylpropiothetin, to homocysteine. Dimethylthetin was ten to twenty times as effective a methyl donor as betaine in tissue homogenates. The transmethylases for these two substances were not the same enzyme, but were distinguishable by a difference in stability at pH 4.

J. A. Muntz (*J. Biol. Chem.* **182,** *489 (1950)*) synthesized choline containing an excess of N^{15}. This was allowed to react with homocysteine in a rat-liver homogenate, and unlabeled dimethylaminoethanol and dimethylglycine were added as carriers. If choline donated a methyl group in the sense of a reversal of its formation, dimethylaminoethanol should be a product. Reisolation of the dimethylaminoethanol, however, recovered none of the labeled nitrogen. On the other hand, the reisolated dimethylglycine contained large excesses of the isotope. This is the product to be expected if betaine donates a single methyl group. The anaerobic conditions observed would be expected to show some nonoxidative methyl donation if choline were subject to such a reaction.

G. L. Cantoni (*Fed. Proc.* **9,** *158 (1950)*) has studied the transfer of a methyl group from methionine to nicotinamide by liver extract. Betaine was an active donor only in the presence of homocysteine, indicating presumably that the transfer occurred by

way of methionine. The reaction was considered to require energy to drive it, and to utilize the energy of adenosinetriphosphate or phosphoglyceric acid. The presence of oxygen was not necessary. H. Borsook and J. W. Dubnoff (*J. Biol. Chem.* **169,** *247* (*1947*); **171,** *363* (*1947*)) had shown earlier that the transfer of methyl groups from methionine to guanidoacetic acid and nicotinamide appeared to require energy. Cantoni achieved a degree of purification of the enzyme, which he proposed to call nicotinamidemethylkinase.

The foregoing experiments indicate that there are specific methyl donors for each methyl acceptor and specific catalysts for each transfer. No two methyl donors can be considered equivalent.

As to the fate of the labile methyl groups, their loss from the organism as creatinine, creatine, and N′-methylnicotinamide is well known, and creatine formation is considered to be a heavy, steady drain on the methyl economy. C. G. Mackenzie *et al.* (*J. Biol. Chem.* **180,** *99* (*1949*)) synthesized methionine containing C^{14} in the methyl group. When 200 mg. were fed to a rat, during the first day one-fourth of the radioactivity was recovered as expired carbon dioxide; the loss continued at a slower rate. Nothing is known as to the factors which govern the loss of these methyl groups from various substances. Such factors may be of great nutritional importance. P. Siekevitz and D. M. Greenberg (*Fed. Proc.* **9,** *227* (*1950*)) have reported that the methyl groups of methionine and choline give rise to formate and can serve as a source of the beta-carbon of serine.

Finally, the question arises as to whether the limitation in the synthesis of labile methyl groups is an absolute or a relative one. In reports presented before the American Chemical Society in September 1949, and before the American Society of Biological Chemists in April 1950, J. A. Stekol *et al.* (*Fed. Proc.* **9,** *234* (*1950*)) reported that rats 30 days old or older could grow on a diet free of all known methyl donors, if vitamin B_{12} and homocysteine were present. On a diet containing ample methionine as the sole sulfur-amino acid, but free of vitamin B_{12}, poor growth was obtained. Addition of vitamin B_{12} gave good growth. E. L. R. Stokstad *et al.* (*Ibid.* **9,** *235* (*1950*)) reported that homocysteine, as a substitute for methionine, gave a growth response in chicks on a diet containing dimethylaminoethanol, but only when vitamin B_{12} was present in the ration. These results suggest that vitamin B_{12} may permit addition of a methyl group to homocysteine without any known methyl donor.

Earlier, pteroylglutamic acid had been rather indefinitely implicated in the metabolism of one-carbon units. W. Shive *et al.* (*J. Am. Chem. Soc.* **69,** *725* (*1947*)) had suggested that para-aminobenzoic acid or a derivative was involved in the introduction of a one-carbon unit to complete the purine ring. The demonstration that formylfolic acid was more active than pteroylglutamic acid itself in overcoming the inhibition of *Streptococcus faecalis R* by 7-methylfolic acid (M. Gordon, J. M. Ravel, R. E. Eakin, and Shive, *Ibid.* **70,** *878* (*1948*)) gave some support to this suggestion. B. Kelley and J. R. Totter (*Fed. Proc.* **9,** *189* (*1950*)) have observed that rats suffered fatty livers on a diet containing 10 per cent glycine. A choline deficiency was believed to be involved, because the kidneys showed increases in weight. The fatty liver was entirely prevented by inclusion of 5 mg. of pteroylglutamic acid in the diet.

Evidence of labile methyl synthesis which seems more conclusive has been reported by W. Sakami (*Fed. Proc.* **9,** *222* (*1950*)) and by A. D. Welch and Sakami (*Ibid.* **9,** *245* (*1950*)). A study of the metabolism of acetone containing a C^{14}—labeled methyl group showed that formate was one of the products. When methyl-labeled acetone was administered to the rat, and the choline and methionine degraded by specific procedures, a portion of the labeled carbon was recovered in the labile methyl groups. A similar recovery of labeled methyl groups was observed

when rat-liver slices were incubated in the presence of C^{14}-labeled formate, homocysteine, dimethylaminoethanol, folic acid, and vitamin B_{12}.

The apparent absence of homocysteine as a constituent of natural diets may or may not diminish the immediate practical importance of the above observations. These developments will certainly be watched with great interest, not only because of the importance of methyl metabolism, but also because of the possible explanation of the biologic role of the two new antianemic factors that may be provided.

DIET THERAPY IN PSYCHONEUROTIC PATIENTS

The possibility that diet might be of benefit in patients having illnesses ordinarily classified as neuropsychiatric seems to have been incompletely explored, although several connections between mental illness and nutrition are known. It is common knowledge that many patients with mental illness have perverted appetites. Some refuse food entirely. Not infrequently such patients develop frank nutritional deficiency. The incidence of mild deficiencies may be very high (*Nutrition Reviews* **8,** *133* (*1950*)). It is recognized that abnormal psychologic factors are of great importance in the overeating which leads to obesity (*Ibid.* **8,** *18* (*1950*); H. Bruch, *Bull. N. Y. Acad. Med.* **24,** *73* (*1948*)). On the other hand, certain vitamin deficiencies, thiamine and niacin for example, may produce definite mental changes (*Nutrition Reviews* **8,** *234* (*1950*)).

S. A. Portis (*J. Am. Med. Assn.* **142,** *1281* (*1950*)) has reemphasized another application of diet therapy in "psychoneurotic" patients. He believes that there is a well-defined group of such patients with abnormal carbohydrate metabolism (Portis and I. H. Zitman, *Ibid.* **121,** *569* (*1943*); F. Alexander and Portis, *Psychosomat. Med.* **6,** *191* (*1944*)) who can be returned to a near normal status by the combined use of diet, atropine, and psychotherapy. The psychologic features characteristic of this condition are apathy, loss of zest, a general let-down feeling of aimlessness, and revulsion against the routine of everyday life. The apathy is seldom connected with anxiety or a pronounced depression. Fatigue is prominent. Such patients often feel fatigued on awakening, become somewhat more so as the morning progresses, temporarily improve after lunch, but again are fatigued by midafternoon. There is practically always complete relief after a heavy evening meal. Along with this chronic fatigue there may be acute attacks of extreme weakness, tremulousness, sweating, and vertigo. The blood sugar is usually only slightly lower than normal under fasting conditions but shows a very flat curve after administration of glucose intravenously.

Intravenous glucose tolerance curves were obtained on 929 patients having complaints in which psychogenic factors played a more or less predominating role. Of these patients, 157 had a flat blood sugar tolerance curve. Apparently most or all of these 157 patients complained of unusual fatigue. How many of the 772 patients with normal glucose tolerance curves also had fatigue is not stated. Portis does not present data to show how consistently abnormal fatigue and flat tolerance curves were associated, nor does he indicate whether the flat tolerance curves were consistently maintained over a period of time in individual patients. Thorough physical and routine laboratory examinations were done in all cases to rule out organic lesions that might account for the abnormal glucose tolerance and the fatigue.

The fatigue patients were treated by the combined use of diet, atropine, and psychotherapy. A diet low in free sugar was fed in three meals daily with snacks of milk or milk and toast in the midmorning, midafternoon, and at bedtime. Special attention was

given to provide a heavy breakfast. The foods allowed and the suggested menu would provide a very adequate diet relatively high in protein and starches but low in fat and readily absorbable sugars. Foods to avoid were cakes with icing, sugar, candy, pie, pastry, jelly, honey, syrup, sweetened fruit juices, alcoholic and carbonated beverages, fat meat and fish, all fried foods, and certain vegetables such as raw onions, radishes, cucumbers, or sauerkraut. Atropine was given four times daily in doses of 1/75 to 1/200 grain with each meal and at bedtime. The doses were adjusted in each patient to provide maximum effect without undue dryness of the mouth or visual disturbances. No details of the psychotherapeutic management were given.

Improvement in these patients was reported to be prompt and striking. Their psychologic status improved and the period of psychotherapy was shortened. As the fatigue improved the doses of atropine were gradually reduced. After periods of therapy from six months to two years, therapy could be eliminated in at least some of the patients with maintenance of a near normal status. Glucose tolerance curves after cessation of therapy were higher than before and in many patients were practically normal.

The exact etiology and pathophysiology of this fatigue syndrome are not clear. Portis believes such patients have an overactive vagus nerve and a "relative" hypoglycemia, the activator in the process being nervous strain and emotional influences. Some support for this view was obtained by doing intravenous glucose tolerance curves on 15 of his patients before and after atropinization. The blood sugar curves after atropine were much closer to normal than before.

Two fatigue patients were subjected to subtotal gastrectomy and high-infradiaphragmatic vagectomy for associated peptic ulcer. Their previously flat tolerance curves became normal following this operation. These results might be explained by vagus overactivity leading to some degree of hyperinsulinism, or to some other upset in the normal glucose regulatory mechanism. It should be noted that these patients did not show a true hypoglycemia, their average fasting blood sugar being approximately 80 mg. per cent. Portis believes that this level of blood sugar is insufficient for these patients and actually represents a "relative" hypoglycemia since measures which raise the blood sugar produce clinical improvement. It is possible too that these patients might have shown considerably lower blood sugar values if blood glucose had been determined during their periods of maximum symptoms rather than under standard fasting conditions.

The syndrome described by Portis seems to be similar in most respects to the well-known spontaneous hypoglycemia which has been described and studied by many investigators. In this sense the present report contributes little that is new. However, Portis does advance a system of management which seems to be successful not only in controlling the disease but in producing lasting benefit after therapy is stopped. Perhaps more important, he reemphasizes the point that among the large group of patients whose symptoms are "psychoneurotic" there are many who have a definite physiologic abnormality which can be recognized, treated, and the patient returned to a useful life. It is all too common for physicians to regard anyone with psychoneurotic symptoms as a rather hopeless case for which little can be done once organic disease is ruled out. However, here is one instance where such patients have a perfectly understandable physiologic abnormality that can be improved by ordinary therapeutic means. Whether these patients are psychoneurotic because of the disturbance in carbohydrate metabolism or whether the mental disturbances produce the carbohydrate changes is not clear. From the practical standpoint it makes little difference.

UPTAKE OF LABELED AMINO ACIDS BY TISSUE PROTEINS *IN VITRO*

A constant interchange in the intact animal of the amino acids in the proteins with free-amino acids was demonstrated in 1939 by Schoenheimer and his colleagues by isotopic labeling. The time for half-replacement of the proteins in the rat has been estimated at seventeen days, in man at about eighty days (D. B. Sprinson and D. Rittenberg, *J. Biol. Chem.* **180,** *715 (1949)*). The rate of protein nitrogen replacement appears to be about a gram per day in man and about 0.2 g. per day in the rat. The visceral organs are intensively involved in this replacement; the muscles to only a minor degree.

It is perhaps not unexpected that this ability of taking up amino acids is rather well preserved in tissues removed from the animal organism and suspended in suitable solutions. Several groups have studied the behavior of isolated tissues, analyzing for the labeled atom in the material precipitated by trichloroacetic acid and prepared by various procedures for analysis. Whether the uptake of the label into these preparations actually represents the introduction of the amino acids into regular positions in the protein molecule, will be considered below.

J. Melchior and H. Tarver (*Arch. Biochem.* **12,** *301, 309 (1947)*) prepared cystine and methionine containing S^{35} and studied the incorporation by liver slices. They observed that labeled cystine was unsatisfactory for this purpose, because any uptake by peptide bond formation was masked by a large uptake due to formation of disulfide bonds. A small uptake of labeled methionine was observed with liver slices, but none with homogenates. The assumption was made that any bound noncystine sulfur represented an uptake of methionine. The uptake was diminished if unlabeled homocysteine was also added.

C. B. Anfinsen, A. Beloff, A. B. Hastings, and A. K. Solomon (*J. Biol. Chem.* **168,** *771 (1947)*; **179,** *1001 (1949)*) suspended rabbit-liver slices in a high-potassium Krebs-Ringer solution and introduced labeled bicarbonate or acetate. In these experiments the tissue had to synthesize its own radioactive amino acids by fixation of carbon dioxide or acetate. The fixation was predominately into the carboxyl groups of aspartic and glutamic acids, released by the action of ninhydrin. There was no incorporation in the absence of oxygen and the extent of incorporation was markedly diminished by mincing the tissue.

By the action of glutamic decarboxylase of squash, the carboxyl group of glutamic acid was shown to be of the same activity as the two carboxyl groups of aspartic acid, both of which are released by ninhydrin. The guanidine carbon of arginine appeared to exchange with the carbon dioxide of the medium. No effect on incorporation was observed upon the addition of glutamate, aspartate, glutamine, asparagine, malate, pyruvate, or alanine.

I. D. Frantz, R. B. Loftfield, and W. W. Miller (*Science* **106,** *544 (1947)*) and P. C. Zamecnik, Frantz, Loftfield, and M. L. Stephenson (*J. Biol. Chem.* **175,** *299 (1948)*) at the Massachusetts General Hospital used carboxyl-labeled DL-alanine and glycine. They compared the incorporation into the proteins of slices of rat hepatoma nodules (induced by dimethylaminoazobenzene) with the incorporation in the normal portions of the same livers. Alanine was incorporated by the malignant tissue several times as rapidly as by the control portions. Most of the alanine was incorporated in the form of alanine itself. These workers released the labeled carbon dioxide by the action of ninhydrin. Very similar results were obtained with labeled glycine. Slices of fetal liver showed rates approaching those shown by hepatomas.

These authors concluded that a difference in rates of *protein degrading processes*, no matter how great, could not account for the higher percentage of radioactivity in the

hepatoma protein. This conclusion is in variance with that of D. Rittenberg and D. Shemin (in D. E. Green, "Currents In Biochemical Research," *p. 272, Interscience Publishers, Inc., New York (1946)*), who concluded that decreases in *catabolic* rates accounted for the net anabolism of neoplasms.

I. D. Frantz, P. C. Zamecnik, J. W. Reese, and M. L. Stephenson (*J. Biol. Chem.* **174,** *773 (1948)*) found that incorporation of alanine was inhibited by very low concentrations of 2,4-dinitrophenol. This reagent is believed to uncouple high-energy phosphate formation from respiratory metabolism (W. F. Loomis and F. Lipmann, *Ibid.* **173,** *807 (1948)*) so that the oxidative energy is not conserved.

T. Winnick, F. Friedberg, and D. M. Greenberg (*Arch. Biochem.* **15,** *160 (1947)*) observed the incorporation of labeled glycine into slices of rat intestine suspended in Krebs-Henseleit medium. The incorporation was inhibited by boiling the preparation or by the presence of azide.

H. Borsook and collaborators (*Fed. Proc.* **8,** *589 (1948)*; *J. Biol. Chem.* **174,** *1041 (1948)*) have described the incorporation of the labeled carbon of bicarbonate, DL-alanine, DL-methionine, L-leucine, L-lysine, and glycine into various tissues. The uptake was fastest with bone marrow cells. The rates for liver slices and diaphragm were about equal. Borsook confirmed the results of Zamecnik as to the rapid incorporation by slices of hepatoma and fetal liver. Borsook has calculated that this turnover rate observed for leucine in bone marrow cells was more rapid than that seen in the intact liver of the rat by Schoenheimer. Borsook found, in agreement with Anfinsen, that mechanical damage to cells reduced the rate of uptake of labeled amino acids.

There are some objections which can be raised to the foregoing results, and these objections become very important when considering the incorporation in cell-free systems. The foregoing workers have not analyzed proteins for the labeling isotope; in fact it cannot be said that their analyses have been of *proteins* at all, but of a collection of molecules that are precipitated by such agents as trichloroacetic acid. Numerous washings have been used; Zamecnik washed his minced slices with solutions of unlabeled alanine to dilute out any adsorbed isotope. The use of ninhydrin for degrading carboxyl-labeled amino acids gave the results additional specificity, especially since this reagent failed to release radioactive carbon dioxide from the preparations until they had been acid-hydrolyzed. This circumstance did not, however, prevent confusion between the incorporation of glycine into phosphatidyl serine and into protein.

T. Peters and C. B. Anfinsen (*J. Biol. Chem.* **182,** *171 (1950)*; *Fed. Proc.* **9,** *214 (1950)*) have avoided these objections to some extent by isolating relatively homogeneous proteins. Chicken-liver slices were shown to incorporate radioactive carbon dioxide into a protein that was separated by alcohol fractionation. This protein gave sedimentation, electrophoretic, and immunologic evidence of being serum albumin. Using a rabbit antiserum specific to chick serum albumin, they were able to demonstrate a net increase of the albumin in the system during incubation. Most of the new albumin was found in the suspending medium.

C. B. Anfinsen (*Fed. Proc.* **9,** *145 (1950)*) has also incubated slices of beef pancreas in medium containing isotopic bicarbonate, and after two hours crystallized radioactive ribonuclease, following the procedure of Kunitz. The isolated enzyme was diluted with five times crystallized unlabeled ribonuclease and recrystallized further. Measurement of the nitrogen and the labeled carboxyl groups gave nearly identical solubility curves, the amount of the solid phase being varied.

A tremendous gain in knowledge of carbohydrate metabolism has come with the discovery of phosphorylase and the manner

in which glucose is polymerized into glycogen. Even greater importance would be attached to a parallel demonstration of the building-up of amino acids into proteins in cell-free systems, because here the order of the amino acid residues presents far greater complexities. Two laboratories, that of Greenberg at Berkeley and that of Borsook at the California Institute of Technology, have reported the incorporation of various amino acids by homogenates of liver. These, homogenates were supposed to be cell-free, or nearly so.

D. M. Greenberg, F. Friedberg, and T. Winnick, and co-workers (*J. Biol. Chem.* **171,** *441* (*1947*); **173,** *437* (*1948*); **175,** *117*, *127* (*1948*); *Arch. Biochem.* **21,** *235* (*1949*)) found glycine to be taken up by such homogenates in appropriate mediums, but not from distilled water. The incorporation was dependent on respiration. It was more rapid with homogenates of fetal liver. Only a small fraction of the label was incorporated as glycine itself; 60 per cent was in the form of serine, and phosphatidyl serine was subsequently found to account for a major part of the incorporation. Subsequently it was also observed that incorporation of the isotopic label was partially accounted for by disulfide links. Treatment of the protein precipitates with fat solvents and mercaptoethanol was introduced to eliminate these types of "quasi-incorporation." Greenberg's group observed the incorporation of carboxyl- and alpha-labeled glycine, alpha-labeled phenylalanine and serine, and methyl-labeled methionine by cytoplasmic granules separated by centrifugation (T. Winnick, *Fed. Proc.* **9,** *247* (*1950*)). The addition of adenosinetriphosphate, magnesium ion, citrate, and a mixture of several amino acids was strongly stimulatory. However, only the label introduced as phenylalanine was entirely resistant to the action of mercaptoethanol and performic acid.

Another method of detecting "quasi-incorporation" was discovered by A. Keston (*unpublished results*). Treatment of protein preparations with dilute alkali led to the loss of a major portion of the label incorporated as carboxyl-labeled glycine.

H. Borsook and colleagues (*J. Biol. Chem.* **179,** *689* (*1949*); *Fed. Proc.* **8,** *589* (*1949*); **9,** *154* (*1950*)) observed incorporation of carboxyl-labeled glycine, L-leucine, and L-lysine by liver homogenates. The incorporation was strongly inhibited by anaerobiosis, arsenite, arsenate, azide, and dinitrophenol; except that lysine was incorporated in some atypical fashion, so that it was scarcely inhibited by any of these factors. L-Lysine was taken up much faster than D-lysine.

The fraction sedimented by centrifugation at 2500 G. consisting largely of mitochondria was most active in the uptake of glycine; the microsome fraction separated at 30,000 G. was much less active. Borsook has referred to incorporation by the supernatant from this centrifugation but data have not been presented.

Borsook has also demonstrated an apparent stimulation of incorporation by cobalt. The extra amino acid was, however, still reactive to ninhydrin, apparently being bound as a metal complex.

During the foregoing experiments, it should be pointed out, probably all the amino acids were present in the free state in appreciable concentrations, whether they were added or not, because of proteolytic activity. Very little attention has been given to the amino acids released in these experiments. The net change in all cases has probably been a decrease in the protein content of the system.

Borsook has sought to demonstrate the reality of incorporation into the peptide chain of protein in a different way. When leucine-labeled protein was partially hydrolyzed by acid at 38°C., a complex peptide fraction containing at least seventeen amino acids could be separated. This fraction included 90 per cent of the radioactivity. Fur-

ther hydrolysis yielded all the radioactivity in leucine. If resolution into a number of individual labeled peptides could be demonstrated by chromatography, this line of evidence would be strong, especially if the position of the labeled amino acid could then be established by a specific peptide degradation.

The formal requirement for proof of incorporation of a specific amino acid into protein has never been attained, not even in Schoenheimer's work, and probably never will be: namely, that administration of a labeled chemical substance must lead to the appearance of the label in another homogeneous chemical entity. A complete demonstration of homogeneity of protein is probably not possible. In the intact animal so large a fraction of the administered amino acid appeared in the protein fraction, and so much of the amino acid in the protein had been turned over, that here any objection is largely formalistic. In homogenates, however, the exchanges are considerably less than 1 per cent of the amount of either the free or the combined amino acid. Under these conditions the results are far more sensitive to binding of the label by reactions of different significance than genuine entrance into the peptide chain. These "quasi-incorporation" reactions appear in certain cases to be enzymatic in character and may have their own interest.

The comprehension of protein synthesis is of such central importance that the difficulties should not be discouraging. Ways should be sought for making the observations more rigorous: (1) by separating protein of as homogeneous a character as possible, (2) by treating the protein with reagents which will eliminate, as far as possible, any isotope introduced by side-reactions, and (3) by degrading the protein in such a fashion as to demonstrate entrance of the label into peptide linkage in several positions in the protein molecule.

ASCORBIC ACID AND DESOXYCORTICOSTERONE IN RHEUMATIC DISEASE

The report on the activity of cortisone (compound E) in alleviating the effects of rheumatic disease is a contribution of great importance (P. S. Hench, E. C. Kendall, C. H. Slocumb, and H. F. Polley, *Proc. Staff Meetings Mayo Clinic* **24,** *181* (*1949*)). Of additional interest is the observation that the simultaneous administration of ascorbic acid and of desoxycorticosterone has an equally beneficial effect (E. Lewin and E. Wassén, *Lancet* **II,** *993* (*1949*)).

Nine patients with mild or severe rheumatic disease received intramuscular injections of 5 mg. of desoxycorticosterone acetate ("doca") and within five minutes an intravenous injection of 1 g. of ascorbic acid (10 ml. of a 10 per cent solution). The authors report that in all patients articular pain decreased and mobility increased immediately and that within fifteen to thirty minutes mobility reached the maximum permitted by the existing anatomic joint abnormalities and muscular atrophy. It was stated that the effects lasted two to six hours but the period of benefit increased in length with successive injections. Ascorbic acid was ineffective when administered orally and the combined administration was ineffective when more than two hours elapsed between injections of the two compounds. No changes in blood pressure or in serum sodium or potassium levels were noted.

Within a few weeks after publication of the above observations by Lewin and Wassén, the value of the treatment was denied in numerous reports on patients in whom little more than euphoria was found (J. H. Kellgren, *Lancet* **II,** *1108* (*1949*); S. J. Hartfall and R. Harris, *Ibid.* **II,** *1202* (*1949*); V. L. Hart and F. Starer, *Ibid.* **II,** *1202* (*1949*); T. D. Spies, R. E. Stone, E. de Maeyer, and W. Niedermeier, *Ibid.* **II,** *1219* (*1949*); E.

Fletcher, B. Lush, J. F. Buchan, and S. Wolff, *Ibid.* **I,** *94* (*1950*)). On the other hand, confirmation of the value of the treatment has appeared (G. E. Loxton and D. LeVay, *Ibid.* **II,** *1134*, *1204* (*1949*); **I,** *209* (*1950*); M. Landsberg, *Ibid.* **I,** *134* (*1950*); J. A. Robertson, *Ibid.* **I,** *134* (*1950*); F. Nashat, *Ibid.* **I,** *135* (*1950*)).

On the assumption that the combined effectiveness of "doca" and ascorbic acid may have been due to the oxidation of the steroid by ascorbic acid, methylene blue (8 ml. of 5 per cent solution, intravenous) has been substituted for the vitamin with equally beneficial results on patients (L. Hallberg, *Lancet* **I,** *351* (*1950*)). The only side reactions following the injection of methylene blue were a bitter taste in the mouth and a pronounced cyanosis which disappeared in a few minutes. The effect was of shorter duration because the amount of methylene blue used had only one-fifth the oxidizing power of the ascorbic acid it replaced. A slight but undoubted objective improvement was noted after administration of either ascorbic acid or of methylene blue alone. Hallberg interpreted these results as an indication of the oxidation of "doca" by ascorbic acid or by methylene blue to yield a product with a physiologic response similar to that of cortisone.

Administration of adrenocorticotropic hormone (ACTH) is also of marked benefit in rheumatic disease, presumably because it stimulates the production of active hormones such as cortisone. It is pertinent that ACTH reduces the level of ascorbic acid in the adrenal gland, an assay method for ACTH being based on this phenomenon (M. A. Sayers, G. Sayers, and L. A. Woodbury, *Endocrinology* **42,** *379* (*1948*)). Hallberg believes that the function of ascorbic acid in the adrenals may be the oxidation of steroids into active hormones.

Studies on experimental arthritis in rats report a beneficial effect of the combined injections of "doca" and of ascorbic acid (G. Brownlee, *Lancet* **I,** *157* (*1950*)). Injection of formaldehyde into the paws of these animals brings about an acute hyperemic and edematous response within two to three days, followed by a chronic reaction which is slow in onset and which results in an enlargement of the ankle joint (H. Selye, *Brit. Med. J.* **2,** *1129* (*1949*)). The severity of formaldehyde-induced arthritis was determined by measurement of the linear cross section just below the ankle. The daily subcutaneous injection of "doca" (1 mg.) and the intraperitoneal injection of ascorbic acid (20 mg.) protected both normal and adrenalectomized animals against the effect of the formaldehyde. Ascorbic acid alone was slightly protective in normal but not in adrenalectomized animals. "Doca" alone exacerbated the arthritic condition in normal rats but was relatively ineffective in adrenalectomized animals.

Certainly far more extensive studies, and repeated confirmation, are necessary before it can be determined that ascorbic acid and desoxycorticosterone have the objective effect described by Lewin and Wassén, particularly in view of the subjective improvement which was noted.

Although present reports are confusing, the lack of agreement on the value of "doca" and ascorbic acid may be due to variations in the types and in the metabolic states of the subjects treated. The supporting data of Hallberg and of Brownlee are suggestive of a positive action of these two dissimilar substances. Furthermore, there appears little doubt that steroid metabolism varies from normal in rheumatic disease, as illustrated by a recent observation that patients with rheumatoid arthritis excreted twice as much urinary pregnanediol following administration of progesterone as was excreted by normal control subjects (I. F. Sommerville, G. F. Marrian, J. J. R. Duthie, and R. J. G. Sinclair, *Lancet* **I,** *116* (*1950*)).

The function of ascorbic acid in the adrenal gland has remained obscure since its recognition in this tissue as "hexuronic acid"

(A. Szent-Györgyi, *Biochem. J.* **22,** *1387 (1928)*). The studies cited in this review may represent promising leads for fruitful investigations of the metabolism of both cortical hormones and the antiscorbutic vitamin.

CARBOHYDRATE METABOLISM IN POTASSIUM DEFICIENCY

Clinical experiences have demonstrated the frequent association of potassium deficiency with diabetic acidosis. Before initiation of therapy serum concentrations of potassium are often increased. A precipitous decrease to very low levels occurs as treatment is begun, after which there is gradual return of serum potassium to normal concentrations. These changes have been ascribed to mobilization and depletion of intracellular potassium, dilution of body fluids with administration of parenteral fluids containing no potassium, and loss of this electrolyte in urine during periods of insulin-lack (*Nutrition Reviews* **5,** *315 (1947)*; **7,** *296 (1949)*).

Certain reactions involved in the intermediary metabolism of carbohydrate and the rates of these reactions are known to be dependent on the presence of potassium in isolated systems. However, little evidence is available to indicate that potassium has an over-all effect on carbohydrate metabolism in the intact animal. L. I. Gardner and coworkers (*J. Lab. Clin. Med.* **35,** *592 (1950)*) discuss the results of experiments designed to assess the effects of potassium deficiency on the metabolism of glycogen and glucose in rats.

The animals used were male albino rats of the Sprague-Dawley strain which weighed 160 to 175 g. when first placed on the experimental diets. Two diets were used. The amount of sodium and calcium contained was the same in each. The first diet, however, contained only 0.07 mM potassium per 100 g., whereas in the second or control diet potassium was present at a concentration of 3.80 mM per 100 g. Oral glucose tolerance tests were done at intervals, as was determination of total eosinophil count. Groups of animals were killed after periods of forty to sixty days and ninety to a hundred and twenty days on the deficient diet. The rats were fasted, then fed glucose as in the glucose tolerance test four hours prior to being killed. Aliquots of liver, skeletal muscle, and blood were taken for analysis of various electrolytes, water, tissue glycogen, and blood glucose. Analyses were done by standard methods. The weight of the adrenals was also determined at the time of death.

Interesting differences in tissue electrolyte patterns in skeletal muscle and liver were demonstrated after animals had been on the potassium-deficient diet for forty to sixty days. In skeletal muscle there was a decrease of 35 per cent in the concentration of potassium. Sodium was increased by more than 100 per cent and there were smaller increases in calcium and magnesium. Even after ninety to a hundred and twenty days on the deficient diet there was no diminution in liver potassium. However, sodium in the liver was increased by 75 per cent, while concentrations of calcium and magnesium rose slightly.

Although levels of chloride in muscle and liver remained unchanged in severe potassium deficiency, serum chloride was decreased from 100 mEq. per liter in normal animals to 81 mEq. per liter in deficient animals. These data indicate that the low level of dietary intake of potassium produced important changes in electrolyte patterns of muscle, liver, and blood. Apparently concentrations of potassium and sodium in the serum of these animals were not determined. It would seem that a valuable opportunity to compare changes in blood and tissue electrolytes in potassium deficiency was overlooked.

Effects of potassium deficiency on carbohydrate metabolism were assessed by determination of fasting blood glucose, liver, and muscle glycogen, and results of glucose tolerance tests. No animal had a fasting blood glucose greater than 140 mg. per cent, even after a period of ninety to a hundred and twenty days of severe potassium deficiency. Glucose tolerance tests were similar in the control group of rats on the diet containing adequate potassium and in rats ingesting the low-potassium diet for forty to sixty days. However, after an additional period of fifty to eighty days of potassium deficiency, concentrations of glucose in blood remained markedly elevated during the tolerance test. The mean concentration in 6 severely depleted rats was 555 mg. glucose per 100 ml. four hours after glucose was given by mouth.

The concentration of glycogen in liver and muscle was markedly increased after forty to sixty days of potassium deficiency. The mean glycogen concentration of liver in animals on the control diet was 2.47 g. per 100 g. wet weight, which compared with 6.56 g. glycogen per 100 g. wet weight of liver in rats potassium-deficient for forty to sixty days. Comparable values for muscle glycogen were 0.36 g. in control animals and 1.53 g. in deficient animals. It was pointed out above that hyperglycemia after glucose administration occurred in rats which had been kept on the potassium-deficient diet for the longer interval of ninety to a hundred and twenty days. At the end of this period both muscle and liver glycogen were found to be extremely low, apparently the result of failure of glycogenesis.

Evidence was obtained that prolonged potassium deficiency is associated with stimulation of the adrenal cortex. After the first thirty days on the deficient diet the total number of circulating eosinophils began to decrease and these cells were almost totally absent after ninety days. In almost all instances the weight of the adrenals was increased in deficient animals. There is the likelihood that the increased deposition of glycogen in liver and in muscle of rats which had been on the potassium-deficient diet for forty to sixty days was the result of increased secretion of the adrenal glycogenic hormone. If this mechanism is effective in the earlier phases of potassium deficiency, other factors must come into play to preclude glycogenesis after more prolonged deficiency. The authors suggest that alterations in the electrolyte composition of the intracellular fluid itself may account for the defect in glycogenesis and the very low tissue-glycogen concentrations and postprandial hyperglycemia associated with prolonged potassium deficiency. However, the data presented indicate that most profound alterations in electrolyte patterns occurred early in the period of deficiency and that tissue electrolyte changes after the fortieth day were relatively minor. It might be anticipated that defects in glycogenesis would have occurred earlier, if the changes in intracellular electrolytes alone account for inhibition of glycogen synthesis and storage.

The observations of Gardner and associates demonstrate important effects of potassium deficiency on carbohydrate metabolism in the intact animal. The deficiency results in stimulation and hypertrophy of the adrenal glands. Postprandial hyperglycemia and depletion of tissue-glycogen stores occur after prolonged deficiency. Hyperglycemia in diabetic coma may conceivably be importantly related to inhibition of glycogenesis resulting directly or indirectly from intracellular depletion of potassium. These considerations would indicate inclusion of potassium in solutions administered parenterally in treatment of diabetic coma.

ANIMAL NUTRITION IN AUSTRALIA

In 1927 the Council for Scientific and Industrial Research of Australia founded the Division of Animal Nutrition with Professor T. B. Robertson as chief. In 1944 the title of the Division was changed to Biochemistry and General Nutrition. The research activities are carried on in a modern laboratory building on the campus of the University of Adelaide, and in addition, a field station of some six hundred acres is maintained at Glenthorne about eleven miles away. In a recent address (*Proc. Roy. Soc. (London) B* **137,** *18 (1950)*) H. R. Marston, the director, described the organization and work of the Division. Supported by government funds, the Division of Biochemistry and General Nutrition consists of some twenty-four investigators trained in physiology or chemistry, and supporting technical personnel.

Inasmuch as sheep raising is a key industry in Australia and since current information regarding the physiology of ruminants is relatively meager, the main interest of the Division has centered on the nutritional biochemistry of wool production. The fact that wool is a protein immediately focused attention upon two general lines of study: (1) various states of nutrition of sheep and their influence upon the efficiency of utilization of the amino acids of the feed for production of wool and meat, and (2) evaluation of the amino acid content of the vegetable proteins in the forage crops and their variation with soil fertility. The protoplasmic proteins of those orders of higher plants that have been studied, were found to be strikingly similar. The potential cystine content of these proteins is the limiting factor in their low theoretic biologic value (35 per cent) for wool production; actually in carefully controlled experiments with pure-bred Merino sheep, the efficiency with which the amino acids absorbed from the gut are converted into fleece, rarely exceeds 10 per cent. There is evidence that this poor yield of wool can be attributed partly to the limited concentration of protein in the pasturage. It appears that the keratin of wool is an expensive product in terms of biologic transformation, in comparison with the protein of milk, meat, and eggs.

The soil along the South Australian coast is not fertile enough to support more than a limited natural flora. The earliest settlers knew that it was impossible to raise sheep successfully in this region and present-day herders are aware that sheep confined to these tracts become anemic and die within the period of a year. Assuming that the composition of the pasturage reflected a deficiency of nutrients in the soil, a mixture of soluble salts of nickel, cobalt, manganese, zinc, iron, and copper was fed and strikingly favorable results were obtained. By process of elimination it was demonstrated that a severe deficiency of both cobalt and copper exists in the soil and is reflected by malnutrition in the sheep.

When an uncomplicated cobalt deficiency exists, the normal alert attitude of the sheep changes to one of "dull and rheumy-eyed listlessness"; there is a blanching of the buccal and conjunctival mucosa and the skin becomes less flexible and may crack. Death results from inanition, an autopsy revealing little more than hunger edema. The anemia, which in the early stages appears to result from a simple decrease in cell numbers, changes to a macrocytic type as the deficiency endures; the blood picture is suggestive of an aplastic condition of the bone marrow.

In the third generation of rats maintained under careful chemical control on rations containing less than 0.03 parts per million of cobalt, the animals are normal, indicating that this species requires little cobalt compared to sheep. If given thrice weekly, 0.1 mg. per diem is sufficient to maintain sheep

without symptoms. The ruminant appears to have an insistent or wasteful need of cobalt, for marsupials, rabbits, and horses thrive in the deficient areas without dietary supplementation. A therapeutic response in deficient sheep is secured only when the cobalt salt is given by mouth, parenteral administration being ineffective. This suggests, in view of the extremely small size of the known effective dose of the cobalt-containing vitamin B_{12}, that the need of the sheep for elaboration of this antianemic factor is extremely small, but that it is the metabolic demand of the symbiotic microorganisms in the rumen that precipitates the typical syndrome of cobalt deficiency in sheep and cattle. The profound anemia responds more slowly to cobalt supplementation than do the other signs of cobalt deficiency, which indicates that the low-oxygen-carrying capacity of the blood is not the fundamental cause of the debility but rather that it is only a symptom of a more general metabolic defect.

When the needs for cobalt on the deficient pastures are satisfied, another syndrome slowly develops, the result of a lack of adequate intake of copper. The copper balance becomes negative, body reserves are depleted, and the blood copper level drops to less than one-fifth of the normal concentration. By supplementing the deficient ration with successively larger though still subminimal doses of copper, the first metabolic process to suffer is the mechanism concerned with keratinization. A more severe deficiency results in a disordered metabolism of iron as shown by a hypochromic anemia and accumulation of hemosiderin in the tissues, and a very low intake of copper by the ewe during pregnancy leads to nervous dysfunction in the lamb. The latter ranges from complete paralysis at birth to mild ataxia appearing at ages of 6 months or more. The histopathologic picture is that of extensive demyelinization in the ventrolateral column always involving the direct and crossed pyramidal tracts.

The second effect of copper deficiency concerns deterioration in quality of the fleece. The capacity of the follicles to produce wool with normal crimp is impaired, and in black sheep the ability to produce the melanoid pigment is lost. Both these capacities return upon dosage with copper salts. As the proliferating cells at the base of the follicle force themselves through the follicular constriction, the globular protoplasmic protein molecules change to a fibrous shape with concomitant chemical change of sulfhydryl groups to the disulfide linkages characteristic of keratin. Histochemical tests with nitroprusside indicate a great delay in the transformation of the SH-groups to S—S-groups in the wool fibers of the copper-deficient sheep. It is plain that the monetary loss due to copper deficiency arises not only from the reduced weight of the fleece but also from the undesirable properties of the wool fiber with respect to technologic processing.

Through the use of the statistical background of pathology in animals pastured on the deficient soils, not only can the areas showing specific deficiencies be delineated, but the degree of deficiency has been estimated. Of considerable agronomic interest are the observations that whereas a grain crop like oats could not be grown on the mineral-deficient soil, even with the addition of nitrogen fertilizer, supplementation with copper not only permitted some growth of oats but immediately raised the demand of the oat plant for nitrogen. When the latter also was augmented, the oats crop was greatly increased. The material of the report emphasizes the enormous practical value of pure research by experts in cognate fields and also the high productivity possible in a community of scholars.

A NEW FORM OF FOLIC ACID—THE *LEUCONOSTOC CITROVORUM* FACTOR

In seeking an organism suitable for microbiologic determination of the amino acid, alanine, H. E. Sauberlich and C. A. Baumann (*J. Biol. Chem.* **176,** *165* (*1948*)) found that a strain of *Leuconostoc citrovorum* was suitable for this purpose. However, for growth on mediums that normally supply adequate amounts of the known "B-vitamins," and which are similar to those used with other organisms for amino acid assay, this organism required an unidentified growth factor richly supplied by refined liver extracts of the type used in the treatment of pernicious anemia. A relationship of this unidentified substance to folic acid was immediately observed, for addition of large amounts of folic acid produced growth in the absence of refined liver extracts.

As previously observed by others (E. E. Snell, E. Kitay, and W. S. McNutt, *J. Biol. Chem.* **175,** *473* (*1948*)), thymidine also promoted growth of the test organism in the absence of the unidentified growth factor. However, liver extracts promoted much faster and heavier growth than did either folic acid or thymidine, and the response of the test organism to graded amounts of the three substances showed differences in the nature of the growth curve, all of which indicated that the active substance of liver extract differed from both folic acid and thymidine (Sauberlich and Baumann, *loc. cit.*, H. P. Broquist *et al.*, *Proc. Soc. Exp. Biol. Med.* **71,** *549* (*1949*)). Subsequent fractionation of the active principle led to concentrates more active by weight than either folic acid or thymidine (H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.* **181,** *871* (*1949*)). The unidentified factor has been widely referred to as the *Leuconostoc citrovorum* factor, sometimes abbreviated CF. A relationship of CF to folic acid was also suggested by the observation that CF competitively overcame inhibition of *Leuconostoc citrovorum* by aminopterin, an antivitamin related to folic acid in structure (Sauberlich, *Arch. Biochem.* **24,** *224* (*1949*)). Sauberlich further observed (*Fed. Proc.* **8,** *247* (*1949*); *J. Biol. Chem.* **181,** *467* (*1949*)) that rats on a folic acid-deficient diet excreted very little CF in the urine, whereas administration of folic acid with the diet, or by subcutaneous or intraperitoneal injection, resulted in large increases in the excretion of CF. A similar result was obtained in man, and these observations suggested an actual conversion of folic acid to CF *in vivo*.

C. A. Nichol and A. D. Welch (*Proc. Soc. Exp. Biol. Med.* **74,** *52* (*1950*)) have now investigated the possibility of a conversion of folic acid to CF *in vitro* by liver slices. Weanling rats were held on a folic acid-free diet containing succinylsulfathiazole for five weeks, at which time an acute folic acid deficiency was indicated by rapid losses in body weight and severe diarrhea. Liver slices from such deficient animals were incubated for two hours with shaking in Krebs-Ringer phosphate solution with the following additions: (a) none; (b) folic acid; (c) ascorbic acid; and (d) folic acid and ascorbic acid. Following incubation, contents of all vessels were homogenized, heated, filtered, and the filtrates assayed for CF with *Leuconostoc citrovorum*.

Liver slices from rats on a complete diet were compared with those from the deficient animals. The findings were as follows: (1) Livers from folic acid-deficient animals were grossly deficient in CF as compared with normal livers (from 36 to 147 units of CF per gram of liver in the deficient livers as compared with 1710 to 2880 in livers of normal animals). (2) Incubation with folic acid (100 micrograms) increased the CF content of both deficient and normal livers to approximately the same high levels. This increase averaged almost 100 per cent for the normal livers, and from 2000 to over 10,000 per cent for the deficient livers. (3) Incubation with ascorbic acid alone approximately doubled the CF content of both con-

trol and deficient livers. (4) Incubation with folic acid and ascorbic acid resulted in much larger increases in CF than when either supplement was employed alone. In view of the previous demonstration of a close relationship between CF and folic acid and results to be cited below, the conclusion appears inescapable that an enzymatic conversion of some of the added folic acid to CF had occurred, and that this conversion was aided by ascorbic acid. Although readily demonstrable in liver slices, this conversion did not occur in homogenates.

An independent approach to the nature of this derivative of folic acid was made at the University of Texas, where T. J. Bond, T. J. Bardos, M. Sibley, and W. Shive (*J. Am. Chem. Soc.* **71,** *3852 (1949)*) discovered the natural occurrence of one or more substances that were more effective than folic acid itself in counteracting the toxicity of methylfolic acid for *Lactobacillus casei*. Details of their work have not appeared, and it is consequently difficult to appraise it critically, but concentrates of the effective substance, termed "folinic acid," were stated by Bardos, Bond, J. Humphreys, and Shive (*Ibid.* **71,** *3852 (1949)*) to support growth of *Leuconostoc citrovorum* under the conditions described by Sauberlich and Baumann (*loc. cit.*).

"Folinic acid" also supported growth of *L. casei* in the absence of folic acid; by mild acid treatment its CF activity was destroyed, but not its folic acid activity. This suggested that folinic acid and CF were identical, and that the active substance was an acid-labile derivative of folic acid. Finally, W. Shive, T. J. Bardos, T. J. Bond, and L. L. Rogers (*J. Am. Chem. Soc.* **72,** *2817 (1950)*) report procedures for the chemical production from folic acid of one or more substances having the activity both of folinic acid and of CF. By treatment with formic acid and acetic anhydride, folic acid was converted to formylfolic acid. Reduction of the latter with hydrogen over a platinum catalyst in the presence of ascorbic acid yielded a reaction mixture which, when autoclaved with water, possessed high activity. An amount of the product equivalent to 0.01 to 0.04 millimicrograms of folic acid per milliliter of medium sufficed to permit half-maximum growth of *Leuconostoc citrovorum;* this crude product is thus apparently about one million times more active than folic acid on the weight basis in promoting growth of this organism. The chemical nature of the active product is not known; it is presumed to contain a modified pteridine ring attached to the same groupings present in folic acid. It also remains to be determined whether the substance produced in this manner is identical with naturally-occurring CF, but the two substances appear to produce the same physiologic response, and it is highly probable that they are either identical, or very closely related.

The evidence thus appears conclusive that CF is a derivative of folic acid, that it is more active than folic acid for some organisms, and that it can be formed *in vivo* from folic acid by some organisms by a process that is favored by ascorbic acid. It may be assumed that *Leuconostoc citrovorum* carries out this conversion with great difficulty, and hence requires CF preformed for rapid growth. The low activity of folic acid for this organism is thus understandable, as is that of thymidine, since under proper conditions the latter substance replaces folic acid for other related organisms (*Nutrition Reviews* **7,** *49 (1949)*). The facts that *Leuconostoc citrovorum* requires CF preformed rather than folic acid, and that other organisms so far tested utilize CF (or folinic acid) in place of folic acid, imply that this substance more closely resembles the enzymatically active form of the vitamin than does folic acid itself. This is also indicated by the enhanced activity of CF, as compared with folic acid, in overcoming the inhibitory effects of folic acid analogues for certain bacteria.

The analogy of this situation to that previously met in the vitamin B_6 group is striking. In the latter case, pyridoxine was the

first form of the vitamin discovered; this proved to be relatively inactive for many bacteria, but more active forms of the vitamin, later identified as pyridoxamine and pyridoxal, were found to occur naturally, and one of these, pyridoxal, is closely related to the coenzyme form of vitamin B_6 (*Nutrition Reviews* **3,** *72, 343 (1945)*; **4,** *232 (1946)*; **5,** *98 (1947)*). These new forms of the vitamin are active with all organisms so far tested for which pyridoxine is active, and are widely distributed in natural materials (*cf. Ibid.* **5,** *98, 143 (1947)*; **7,** *215 (1949)*). On the basis of the results cited above, it seems probable that some similar relationship exists between folic acid and CF or folinic acid. It has been indicated that more than a single form of the latter occurs naturally (Bardos *et al., loc. cit.*). Detailed testing of such relationships should rapidly become possible with the availability of a synthetic substance with activity.

The finding that ascorbic acid enhances conversion of folic acid to CF both in liver slices and *in vitro* is of great interest, since if this conversion is essential to permit folic acid to carry out its biologic function, as indicated above, a possible explanation for previous observations of a metabolic interrelationship between folic and ascorbic acids would be provided. Examples of such interrelationships, previously discussed (*Nutrition Reviews* **7,** *49 (1949)*), are the effect of folic acid *or* ascorbic acid in reducing the excretion of "tyrosyl" derivatives and keto acids in scorbutic guinea pigs, and the favorable effects reported on administration of ascorbic acid to folic acid-deficient rats.

We may confidently expect continued investigation of these bacterial growth factors to provide results of the greatest importance to an understanding of the role of folic acid, and possibly of ascorbic acid, in the metabolism of both bacteria and animals.

LIPOTROPIC FACTORS, PHOSPHATIDE TURNOVER, AND NITROGEN BALANCE IN CIRRHOSIS OF THE LIVER

The dramatic preventive and causative effect upon the fatty liver disease produced in animals by diets deficient in protein and lipotropic substances such as methionine and choline has led to the use of such lipotropic substances in the treatment of liver disease in man. However, no evidence has yet been presented indicating that addition of these substances to a nutritious diet hastens recovery from liver disease or in any other way alters the prognosis (*Nutrition Reviews* **8,** *13 (1950)*). Nevertheless, there is little doubt that these substances, and perhaps others, are implicated in the deficiency state that occurs in various kinds of nutritional cirrhosis in man (*Ibid.* **8,** *13, 55 (1950)*). Two studies bearing on this subject have been published.

An investigation by W. E. Cornatzer and D. Cayer (*J. Clin. Invest.* **29,** *534, 542 (1950)*) concerns studies of the rate of turnover of phospholipid in normal individuals and patients with cirrhosis of the liver. The authors administered radioactive phosphorus and subsequently determined the specific activity and relative specific activity in the total plasma phosphorus and the inorganic and lipid fractions.

The first group of 13 normal persons showed evidence of phospholipid synthesis in the first twenty-four or more hours following administration of radioactive phosphorus, as measured by the increase in specific activity of plasma phospholipid.

Another group of 6 normal individuals was given choline (to 5) and methionine (to one), and the rate of plasma phospholipid synthesis studied. There was no significant difference in the rate of phospholipid synthesis between the individuals given the lipotropic substances and those who were not. Three of these individuals were again given a single large dose of choline with similar results.

Another group, this time composed of 20 patients with clinical and laboratory evidence of portal cirrhosis, was studied. The initial rate of phospholipid synthesis was measured in 8 patients and the values fell within the range found for the normal individuals previously studied, although they were all in the lower part. After treatment of 5 of these patients with choline and methionine for a few months there was no change in the rate of phospholipid synthesis even though clinical improvement was noted.

In contrast to the normal individuals, a single large dose of choline or of methionine did produce a significant increase in plasma phospholipid synthesis in 7 of 8 patients with cirrhosis of the liver prior to treatment. This "load test" was again performed in these patients following continuous treatment with lipotropic agents for several months, but the stimulation of phospholipid synthesis observed prior to therapy was not seen. The authors conclude from these data that although the rate of phospholipid synthesis in patients with liver disease is not outside of normal limits, it can be stimulated prior to treatment by administration of large doses of choline and methionine. They believe that this indicates that these patients with untreated cirrhosis of the liver had a deficient supply of choline or its precursors.

The second study concerned the effects of choline and methionine and also of testosterone and of increases of dietary protein in patients with chronic liver disease. Alterations in nitrogen balance, particularly, were observed (G. J. Gabuzda, R. D. Eckhardt, and C. S. Davidson, *J. Clin. Invest.* **29**, *566 (1950)*).

The study included 5 patients with cirrhosis of the liver and one with infectious hepatitis. The basal diet to which these agents were added varied from 0 to 100 g. of protein and provided presumably adequate calories. Each patient was continued through several regimens with addition of one or more of these substances and with suitable control periods between each of the study periods.

Although the diets differed greatly in protein content, so that in some instances negative nitrogen balance occurred while in others positive nitrogen balance occurred, the effects of the administered substances were essentially the same in character irrespective of the nitrogen intake. Thus, addition of choline and methionine to the diets did not alter the nitrogen balance except for the slight increase in positivity apparently due solely to retention of the nitrogen contained in these substances. This was further substantiated by an experiment in which glycine in amounts equivalent in nitrogen to the choline and methionine previously administered was given to one patient. Nitrogen balance was again more positive in amounts approximating that of the nitrogen contained in the glycine itself.

Testosterone as the propionate was administered to 3 patients and produced a definite decrease in nitrogen excretion and therefore a more positive nitrogen balance. The effect was not great, however, and disappeared within a few days after administration of testosterone was discontinued.

Four of the 5 patients were given an increase in dietary protein. An increase in positive nitrogen balance occurred in each instance, indicating that the patients were able to utilize the increased dietary protein. The authors concluded that positive nitrogen balance was most effectively and economically achieved by providing adequate quantities of dietary protein rather than by supplementation of the diet with choline or methionine or by intramuscular administration of testosterone.

The results of these two papers, although superficially divergent, are in harmony and indicate, as might be expected from animal experiments, that in patients with cirrhosis of the liver there may be a deficiency of choline or its precursors but that as yet no specific benefit has been shown to occur from addition of these substances to a nutritious diet or from the anabolic effect of testosterone propionate.

IN VITRO DIGESTIBILITY OF SOY PRODUCTS

In contrast to most proteins, that of the legume cereal seeds shows an improvement in nutritional value after heating. The biologic assays, upon which this conclusion is based, with particular reference to soybeans, have been carried out with chicks (W. E. Ham, R. M. Sandstedt, and F. E. Mussehl, *J. Biol. Chem.* **161,** *635 (1945)*) as the experimental animal, as well as with rats (A. A. Klose, B. Hill, and H. L. Fevold, *Proc. Soc. Exp. Biol. Med.* **62,** *10 (1946)*) and mice (R. J. Westfall and S. M. Hauge, *J. Nutrition* **35,** *379 (1948)*). One explanation for this phenomenon rests upon the presence in soybeans of a heat-labile factor with the property of inhibiting the activity of the trypsin of pancreatic juice. This substance, presumably an enzyme, has been crystallized (M. Kunitz, *J. Gen. Physiol.* **29,** *149 (1946)*) and it has been shown that it is a protein exerting its growth-retarding effect when added to an otherwise adequate ration. D. Melnick, B. L. Oser, and S. Weiss (*Science* **103,** *326 (1946)*) had previously stated that the increase in nutritional value of soya protein as a result of heating, was accompanied by an augmentation in the *in vitro* pancreatic digestion of the protein.

The study of M. Simon and D. Melnick (*Cereal Chem.* **27,** *114 (1950)*) was designed to determine in finer detail the relation between the nutritive value and the *in vitro* digestibility of soybean protein and to decide whether the *in vitro* digestibility could be used as a reliable technic for estimating the nutritive value. The products used in the investigation were (1) dehulled soybeans; (2) unheated solvent-extracted soy flakes; (3) bakery-type soy flour, mildly heated to eliminate the bitterness of the raw bean, but not sufficiently heated to impair its baking qualities; (4) a product optimally heated with respect to the nutritive value of the protein; (5) an overheated product.

In vitro digestibility was expressed as the proportion of formol-titratable nitrogen at a given time to the nitrogen obtained by a complete acid hydrolysis of the protein. The antitryptic activity of soybean products was measured by equating the velocity constant of a pancreatin digestion of casein with the soy flour added, with the velocity constant of a similar digestion without the addition of the antitryptic factor. The biologic estimate of nutritive value was made with growing rats and was expressed as grams body weight gained per gram of protein eaten.

With the unheated soya products (samples 1 and 2) it was observed that the antitryptic activity was relatively high and the protein efficiency low; with the bakery flour (sample 3) the antitryptic activity was high but the protein efficiency was greatly increased; with the heated products (samples 4 and 5) the antitryptic activity was low and the protein efficiency again high. Thus, the correlation between antitryptic activity and protein efficiency in these experiments was not clearcut. There was also poor correlation between the extent of *in vitro* digestion and the protein efficiency. In the purposely overheated flour (sample 5), however, a high-protein efficiency, a high extent of digestion, and a very low antitryptic activity in contrast to the data on the more mildly heated products, indicate that other enzymes may be present in the latter. The authors call attention to naturally-occurring proteolytic enzymes in the raw soybean (D. Melnick and B. L. Oser, *Food Technol.* **3,** *57 (1949)*) which, because of their synergistic rather than additive effect on pancreatic proteases, alter the *in vitro* digestibility in such a way as to render interpretation difficult.

The contradictory data lead one to conclude that in soybean products and in other food substances containing inherent enzymes affecting proteolysis, the use of *in vitro* digestibility, in lieu of biologic estimations of nutritive values of proteins, is not justified without further experimentation relative to inactivation of the interfering enzymes.

NOTES

Effect of Starvation on Glucose and Citric Acid Metabolism in Man

Abnormalities of carbohydrate metabolism have been observed in normal individuals and in diabetic patients following alterations in carbohydrate intake and after prolonged periods of starvation. "Starvation diabetes" has been described as the decreased tolerance to orally administered carbohydrate (glucose) during periods of starvation (*Nutrition Reviews* **6,** *221 (1948)*).

In view of the close relationship between the metabolism of carbohydrate and of citrate, S. Natelson, J. B. Pincus, and J. K. Lugovoy (*J. Clin. Invest.* **27,** *446, 450 (1948)*) studied the blood citric acid concentration before and after administration of glucose. They first found that in normal individuals the highest blood citric acid concentration was in the fasting state. Following administration of glucose by mouth there was a drop in the blood citric acid level of significant degree and a gradual return to normal in the course of approximately six hours. The drop in the blood citric acid level was greatest during the time that the rate of reduction in glucose concentration was greatest. Most, although not all, of the diabetic patients studied had a similar response in blood citric acid concentration following oral administration of glucose.

With these studies and similar ones in mind, K. Lundbaek, V. P. Petersen, and F. Schönheyder (*J. Clin. Invest.* **29,** *361 (1950)*) investigated the effect of starvation upon changes in serum citric acid level after oral administration of glucose. Twelve normal students were fasted completely except for water for seven days. Glucose tolerance was then studied following oral administration of 70 g. of glucose. Serum citric acid was concomitantly determined. Control (nonstarved) studies were made in all patients either before the starvation period or several weeks after. All individuals lost weight during the starvation period (average 5.6 kg.) and ketonuria appeared. Ten of the 12 subjects exhibited characteristic diabetic-type glucose tolerance curves after the fast. Two, however, exhibited normal tolerance to orally administered glucose.

In the nonfasting state there was the expected drop in serum citric acid concentration following glucose administration. However, after the period of fasting no drop in citric acid concentration occurred, in fact, often a slight or definite rise in serum citric acid concentration was noted. This reversal of the normal citric acid-glucose relationship occurred in all except 2 individuals who also were the 2 who had relatively normal glucose tolerances at the end of the fast. The serum citric acid response to glucose was normal in these 2 individuals as was the glucose tolerance, despite the prolonged fast.

The authors believe that their results indicate that a diminished carbohydrate utilization appears in most individuals following a prolonged fast, as indicated by intolerance to glucose and failure in the usual fall in blood citric acid concentration. These results were compared with those of K. Lundbaek and J. A. F. Stevenson (*Fed. Proc.* **7,** *75 (1948)*), who found that isolated rat diaphragm muscle took up far less glucose and presumably, therefore, metabolized less glucose following a period of carbohydrate-free diet.

These results further substantiate the decrease in carbohydrate utilization following periods of starvation that had been found clinically.

Energy of Formation of Protein-protein Complexes

No precise data are available on the energy of formation of bonds between enzymes and their substrates. Until such data are obtained constructive speculation on the

nature of these bonds is almost impossible. So far as the antigen-antibody combinations are concerned, only one determination was available until recently. W. C. Boyd *et al.* (*J. Biol. Chem.* **139,** *787 (1941)*), studying the heat produced in the combination of hemocyanin and its horse antibody, found a value of 40,000 calories per mole of antihemocyanin. Interpretation of this result was dependent on arbitrary postulates and proved difficult.

R. Wurmser and S. Filitti-Wurmser (*Biochem. et biophys. acta* **4,** *238 (1950)*) conducted a thorough thermodynamic analysis of the isohemagglutination, a reaction well suited for this type of study because of its complete reversibility and because it is a surface reaction more easily interpreted from a quantitative standpoint.

The effect of temperature on the equilibrium was determined and equilibrium constants at 37 and 25°C. were measured. The heat of the reaction was both calculated and directly determined with excellent agreement between the two values. The variations of free energy and entropy involved in the saturation of a single bond were calculated, and the number of molecules of agglutination per red cell surface was determined and found to be in agreement with experimental results.

Investigations of this kind are difficult, both from the point of view of procedure and correct interpretation of results. But it is from such efforts that the nature of mutual reactions between proteins, one of the fundamental problems of biochemistry, can be understood.

Nutrition in Medicine

Annual Reviews, Inc., Stanford, California have recently published Volume I of *Annual Review of Medicine*. In the introductory chapter of the section on "Nutrition and Medicine" the authors state: "Fundamentally, nutrition is of vital importance for the growth and maintenance of all body cells. It enters into all aspects of health including mental health. Nutrition is concerned with food and the ingredients of food known as nutrients which are necessary for health, with the physiologic action of these nutrients, and with the consequences of not having effective cell concentrations of any of the 50 to 60 nutrients now known. In our present day civilization in which the prevention, control, and treatment of most of the serious infectious diseases are well in hand, nutrition is the single most important environmental factor affecting the health of Americans. Such a statement does not refer in any way to the classical nutritional deficiency diseases of scurvy, beriberi, or pellagra, which are of relatively minor importance in this country today, thanks to the intelligent and prompt application of nutritional findings important to the prevention and treatment of these diseases. But it does refer to the importance of nutrition, including what one might call cellular nutrition, to the so-called degenerative diseases including the cardiovascular renal diseases and cancer; it does refer to the role of nutrition in diseases of the liver and the hematopoietic system; it does refer to the dangers of obesity to good health; and it does refer to the potentialities of antinutrients and antimetabolites in the etiology and treatment of disease. Hence, we (the authors—*ed.*) feel justified in stating that in present day America, nutrition is the single most important environmental factor affecting health."

Recent Nutrition Books

Patterns of Living in Puerto Rican Families. Lydia J. Roberts and Rosa Luisa Stefani. University of Puerto Rico, Rio Piedras, Puerto Rico. 1949. Pp. 411.

The Nutritional Improvement of Life. Henry C. Sherman. Columbia University Press. 1950. Pp. 270. Price: $3.75.

Composition of Foods—Raw, Processed, Prepared. Bernice K. Watt and Annabel L. Merrill. United States Department of Agriculture Handbook No. 8. 1950. Pp. 147.

Human Milk. S. K. Kon and E. H. Mawson. Special Report Series No. 269 (1950). York House, Kinsway, W. C. 2, London. (Post Orders: P. O. Box 569, S.E.1) Price: 4s. 6d. By Post 4s. 9d.